THE PLANT DISEASE REPORTER

Issued By

CROPS RESEARCH DIVISION

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

Volume 44 Number 8

August 15, 1960

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

COMMONWEALTH MYCOLOGICAL INSTITUTE
15 SEP 1960

SUGGESTIONS FOR PREPARATION OF MANUSCRIPTS FOR THE PLANT DISEASE REPORTER

(1) GENERAL: The Reporter page measures 9 inches long with the heading or 8 3/4 inches for the text part, by 6 inches wide. The copy is typed on a larger page, 11 1/4 inches of text or 12 inches overall in length by 8 inches in width, and reduced 25 percent in the photographic process of reproduction. Illustrations or tables larger in either dimension will take a correspondingly greater reduction. Only one size of type is available for text, footnotes, or tables.

(2) MANUSCRIPTS should be the original ribbon copy, not carbons, clearly typed and double-spaced throughout, including tables, footnotes, and bibliographies. (Note -- only one copy is needed.) Footnotes should be typed at the bottom of the page.

(3) ABSTRACTS are requested for all except very short articles.

(4) CAUSES OF DISEASES should be named. For bacteria, fungi, nematodes, etc., give the Latin name of the organism; for viruses either or both the accepted common name of the virus or a Latin name if you prefer it and there is one; for non-parasitic diseases state the causal factor if it is known. If the cause of a disease has not been determined say so.

(5) LITERATURE REFERENCES should be given in alphabetical order and numbered for citation in the text. We follow the AIBS suggestion of placing the year of publication after the author's name. Please check your references carefully since we cannot do it for you. Be sure that text citations and bibliography agree; that foreign-language references are correct; that number or month is cited for periodicals that are not paged consecutively throughout the volume.

(6) NAMES OF FUNGICIDES should be given according to the suggestion of McCallan et al. in Phytopathology (45 (6): 295-302. 1955).

(7) ILLUSTRATIONS should be sent to us unmounted. To prevent mistakes, write figure numbers on the back, and mark the top of each print when necessary. A sketch can show a preferred arrangement but please keep in mind page size, shape, and standard reduction (see above under General), and remember that figure titles and legends are part of the page. Lettering should be clear and large enough to be legible after reducing. Drawings, maps and graphs can be photographs or originals, but should be finished and ready for reproduction, not just sketches.

(8) TABLES should be carefully thought out with particular attention to the Reporter's limitations in reproduction. Make titles and headings definite and self-explanatory. Designate footnotes in tables with superscript lower-case letters. Be sure that text discussion agrees with the data in the table. Do not abbreviate names of crop varieties.

(9) REPRINTS cannot be supplied since there is no way in which we can be reimbursed. However,

(10) The MULTILITH PLATES from which reprints can be made will be sent if requested at the time the article is submitted. The press size of these plates used for the Reporter is designated as small -- maximum image 9 1/2 by 13 inches, maximum paper size 9 3/4 by 14 inches -- for Model 1250. Most of the Experiment Stations have this type of multilith machine.

ACCEPTANCE OF MANUSCRIPTS

The increase in the volume of pertinent material offered for publication in the Plant Disease Reporter has made it necessary to limit the subject matter and the length of articles accepted. The subject matter should emphasize new things in plant pathology, such as new records of disease occurrence, serious outbreaks and epidemics, conditions affecting development of plant diseases, techniques of investigation including instrumentation, new discoveries in control including new materials and their evaluation. Manuscripts will be limited to 12-double-spaced typed pages, including tables, graphs, and photographs. Because of reproduction costs photographs should be kept to a minimum. Insofar as possible, material should be presented as graphs rather than tables. Papers cannot be accepted for publication that report routine control experiments, reviews, bibliographies without annotation, results of routine surveys, mere summaries or lists of plant diseases. By following this procedure we hope to continue publishing all articles promptly.

Paul R. Miller

Manuscripts for and correspondence about this publication should be sent to:

PLANT DISEASE REPORTER
Epidemiology Investigations, Crops Protection Research Branch
Plant Industry Station, Beltsville, Maryland

THE PLANT DISEASE REPORTER

Crops Research Division
Volume 44

Plant Industry Station, Beltsville, Maryland
August 15, 1960
Number 8

CONTENTS

1. History, distribution, races, and disease cycle of Xanthomonas malvacearum in California
W. C. SCHNATHORST, et al. 603

2. Leaf spot of peanut in Georgia caused by Leptosphaerulina arachidicola
E. S. LUTTRELL and L. W. BOYLE 609

3. Distribution and a mode of spread of Cytospora canker in an orchard of the President plum variety in California
LUIGI CHIARAPPA 612

4. Isolation of Olpidium brassicae from roots of lettuce showing big-vein symptoms
JOHANNA T. BARR 617

5. Banana fruit-spot caused by Deightoniella torulosa (Syd.) Ell.
DONALD S. MEREDITH 618

6. An assay for the detection of nematode repellents
D. DAVIS and J. E. DEAK 622

7. Some sap-stain fungi found in Minnesota
ROBERT N. CAMPBELL 625

8. Peach "shot hole" in Arizona -- 1959
PAUL D. KEENER 629

9. Taphrina pruni-subcordatae (Zeller) Mix, causing witches'-broom on Italian and French prunes
JULIUS L. HEINIS 630

10. The virus content of flowering cherries at Summerland, British Columbia
T. B. LOTT and F. W. L. KEANE 632

11. Virus infection not the cause of earliness in three strains of Italian prune
T. B. LOTT, et al. 633

12. Leaf enations in apple inoculated from cherry
T. B. LOTT and F. W. L. KEANE 634

13. Transmissible corky pit of Flemish Beauty pear
F. W. L. KEANE and MAURICE F. WELSH 636

14. Soil inhibits infection with tobacco necrosis virus
C. E. YARWOOD 639

15. Crown rots and Botrytis flower blight of statice
CURTIS R. JACKSON 643

16. The susceptibility of twenty-three tree species to black root rot
SAMUEL J. ROWAN 646

17. A method of evaluating fungicides in the soil under controlled conditions
J. H. REINHART 648

18. Heat-induced stolon rot of Ladino white clover in the greenhouse
R. A. KILPATRICK 653

19. Two serious foliage diseases of western white pine in the Inland Empire
CHARLES GARDNER SHAW and CHARLES D. LEAPHART 655

20. A preliminary report on the use of pimaricin in the isolation of Phytophthora spp. from root tissues
J. W. ECKERT and PETER H. TSAO 660

21. Halo scab--result of an association of Elsinoë fawcetti and Brevipalpus phoenicis destructive to sour-orange seedlings
L. C. KNORR and GINO MALAGUTI 662

22. Botrytis cinerea and Sclerotinia sclerotiorum in seed of safflower and sunflower
W. E. SACKSTON 664

23. Epidemic leaf-blighting of California-laurel
J. R. PARMETER, Jr., et al. 669

24. Control of stripe smut on Poa pratensis with nabam
R. J. LUKENS and E. M. STODDARD 672

25. Cacao canker in Guatemala incited by Ceratocystis fimbriata
EUGENIO SCHIEBER and OSCAR NERY SOSA 672

26. New pine hosts for southern fusiform rust
F. F. JEWELL 673

27. Book Review (Vegetable Diseases and their Control)
PAUL R. MILLER 673

HISTORY, DISTRIBUTION, RACES, AND DISEASE CYCLE OF XANTHOMONAS MALVACEARUM IN CALIFORNIA[1]

W. C. Schnathorst, P. M. Halisky, and R. D. Martin

Summary

Angular leaf spot of cotton was found in California in 1912 and 1929 but did not become permanently established until 1951. There appear to have been at least four separate introductions of the disease into the State. The disease is confined chiefly to the western part of the San Joaquin Valley in central California and is associated with overhead sprinkler irrigation. Eleven isolates of the causal bacterium, obtained from separate field collections and tested on host differentials, all appear to be Race 1 of Xanthomonas malvacearum. The distribution of the disease suggests that contaminated planting seed is involved in its spread. Other evidence indicates that cotton gins may be sources of contamination of otherwise disease-free planting seed. Acid-delinting of cotton seed proved effective in removing externally seed-borne infestations of X. malvacearum.

Angular leaf spot of cotton incited by Xanthomonas malvacearum (E. F. Sm.) Dow., is world-wide in distribution and usual wherever cotton is grown (7, 17). The most recent world-distribution map of X. malvacearum (8) does not indicate its presence in California, seemingly because of the lack of published records. This paper reports on occurrence of the disease in this State, its history, its distribution, the races present, the disease cycle in California, reasons for its recent increase, and possible measures of control.

HISTORY IN CALIFORNIA

Angular leaf spot in California was first observed in October 1912 by R. L. Piemeisal, at Bard, Imperial County, in southern California[2]. It was not observed again until August 1929, when F. W. Herbert and O. F. Cook found it on seedlings at the U. S. Cotton Field Station, near Shafter, in central California[2]. In both instances the disease was apparently introduced on imported seed. There has been no record of it at the U. S. Cotton Field Station since 1929. In September 1951, however, the disease was found near Caruthers, Fresno County, in central California[2]. Since then the disease has occurred each year in various parts of the San Joaquin Valley of California. Evidence that the pathogen was reintroduced in 1959 was obtained when seed of a variety other than Acala 4-42 was planted in the restricted "one-variety" area of the State. Angular leaf spot developed on the imported variety and spread to adjacent Acala plants. Thus, there appear to have been at least four well-documented instances of introduction of the pathogen into California.

Manifestations of the disease in California are the same as elsewhere. The circular translucent lesions on cotyledons, angular lesions on leaves, systemic infection of leaves, black arm phase on the stems, and boll rot have all been observed (Fig. 1).

DISTRIBUTION OF ANGULAR LEAF SPOT

Cotton disease surveys were conducted in 1951-1959 in the San Joaquin Valley. The most extensive surveys were in 1956-1959. Most of the occurrences recorded in the distribution map (Fig. 2) were observed in 1957-1959. The disease is presently confined to five counties: Fresno, Kern, Kings, Madera, and Tulare. It has not been observed in the southern cotton-growing areas since the original observation in 1912.

In 1951 angular leaf spot recurred in the San Joaquin Valley; since then, it has been concentrated on the western slopes of the valley though it has recently spread to other parts of the valley (Fig. 2). Sixty-six occurrences have been recorded since 1951.

---

[1]Contribution from the Crops Research Division, Agricultural Research Service, United States Department of Agriculture; Department of Plant Pathology, University of California, Davis; and California Planting Cotton Seed Distributors, Bakersfield, California.

[2]Personal correspondence between Drs. G. L. Stout, Bureau of Plant Pathology, California State Department of Agriculture, and J. B. Kendrick, Sr., Department of Plant Pathology, University of California, Davis, October 16, 1951. Records obtained from State Department of Agriculture card-index files.

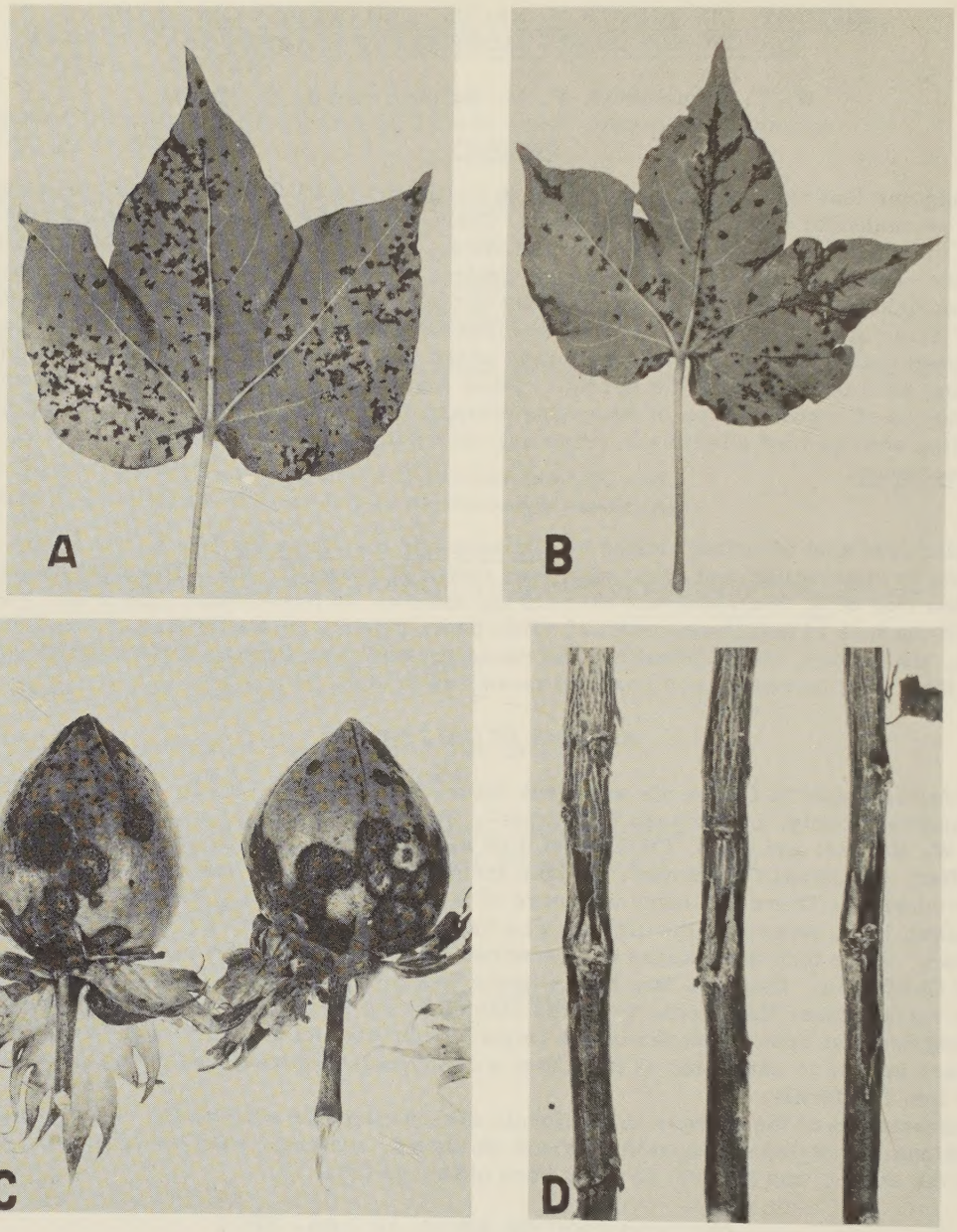

FIGURE 1. Symptoms of bacterial blight on Acala 4-42 cotton in California. A -- angular leaf lesions; B -- systemic leaf infection; C -- lesions on cotton bolls; and D -- lesions on cotton stems known as black arm.

The higher incidence in the western San Joaquin Valley is correlated with widespread use of sprinkler irrigation there. In the eastern portion of the valley most of the fields are furrow-irrigated.

Each occurrence of the disease but one on the map (Fig. 2) is associated with sprinklers. The exception was a furrow-irrigated field flooded by heavy rains when the seedlings were about 2 inches high. In addition, the grower had soaked the seed to hasten germination. The high incidence (32%) strongly suggests that the seed was inoculated during soaking by infested seed in the seed lot.

Sprinkler irrigation for cotton in the San Joaquin Valley is a fairly recent innovation. In the early 1940's sprinklers were introduced into the western portion of the valley. Sprinklers are becoming more common elsewhere in the valley because growers feel that the water use is

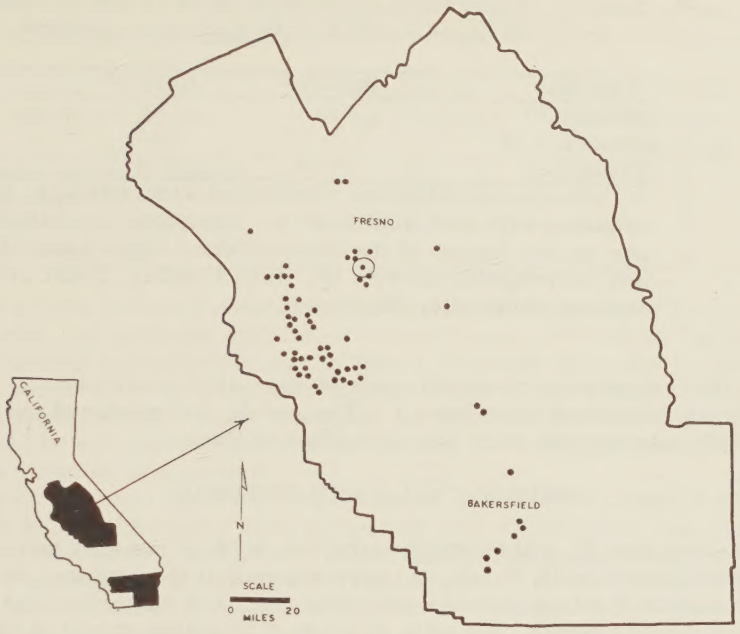

FIGURE 2. Distribution map of angular leaf spot of cotton in California 1951-1959. The blackened areas on the small map represent the cotton-growing regions in California. The enlarged map shows the distribution of the disease in the centrally located San Joaquin Valley. Each dot represents a separate occurrence. The open circle indicates the approximate point of disease origin in 1951.

more efficient and cotton yields are often improved. There is, consequently, a growing concern that the incidence of angular leaf spot may be increased.

RACES OF XANTHOMONAS MALVACEARUM

One of the best methods of controlling angular leaf spot is the growing of resistant varieties. A breeding program, however, requires knowledge of the race or races of the pathogen present. In 1954 Hunter and Blank (11) demonstrated two pathogenic types of X. malvacearum in the United States on the basis of varietal reactions. In 1955 the nomenclature subcommittee designated these two pathogenic types Race 1 and 2 (13). Race 1 is the more prevalent; Race 2 is presently limited to portions of New Mexico and Texas. Studies on races in other parts of the world were reported by Brinkerhoff (3).

Because of evidence of repeated introductions of contaminated cotton seed, it was felt that both races might be present in California. In 1959 infected leaves were collected from nine widely separated fields in western San Joaquin Valley. X. malvacearum was isolated from all collections. Table 1 lists the cotton differential varieties used and their reactions to the two races of the pathogen. Acala 4-42, the predominant California variety, was used as an additional susceptible check. Inoculum was prepared by growing the organism for 3 days on slants of potato-dextrose-peptone agar plus 0.1% $CaCO_3$, flooding the slants with 15 cc of sterile distilled water, and suspending the bacteria by scraping with a sterile glass rod. A sterile DeVilbiss atomizer was used to spray the bacterial suspension onto the leaves. The leaves were sprayed to run off, primarily on the under surface. The inoculations were made between 10:00 and 11:30 a.m., when the stomates in cotton leaves are open (18). Two 7-week-old plants of each differential were separately inoculated with each bacterial isolate. The plants were covered with a polyethylene bag internally atomized heavily with sterile distilled water to produce a high relative humidity. The polyethylene bags were held in place with a rubber band on the rim of each pot. Plants were kept in a greenhouse with an average temperature of 80° F (26° C). Symptoms were evident in 12 days, but the differential reactions were not determined until 21

Table 1. Differential reaction of three cotton varieties to Races 1 and 2 of Xanthomonas malvacearum.

Variety	Race 1[a]	Race 2[a]
Mebane B1	- to +	-
Stoneville 20	-	+++
Texas S-9	++++	+++

[a]- indicates no infection; + indicates susceptibility, increasing with each additional +. Reactions are based in part on the Report of the Nomenclature Subcommittee (13) and correspondence with Dr. John Presley, Plant Industry Station, Beltsville, Maryland.

days had elapsed. Reactions were recorded again 56 days after inoculation.

All nine bacterial collections were Race 1. The isolate that produced the disease in 1951 and one from the 1959 introduction were also identified as Race 1.

DISEASE CYCLE AND CONTROL

Massey (12) showed that X. malvacearum survived on plant remains and infected the succeeding crop. Several workers (4, 9, 10, 15) have shown that the pathogen is seed-borne, and Tennyson (16) demonstrated actual entrance into the seed. The bacterium has been recovered from year-old dried leaves, stems, and bolls of Acala 4-42 cotton stored in this laboratory and from seed taken from infected bolls. Ark (1) isolated the pathogen from dried leaves held in his laboratory for 6 years.

In 1958 the number of cotton fields with angular leaf spot in the San Joaquin Valley increased three- to four-fold. Some of the fields involved had been out of cotton production for several years, one for as long as 12 years. The evidence from one field strongly suggested that the planting seed was contaminated. Since all planting seed used in the San Joaquin Valley is supposedly free of X. malvacearum, an effort was made to determine any sources of contamination.

In California, planting seed is taken only from fields that are furrow-irrigated according to the specifications of the California Planting Cotton Seed Distributors organization. A few fields are contracted for seed in the blight area in western San Joaquin Valley. Some of the cotton gins in this area gin both planting seed and seed from sprinkled fields that may have angular leaf spot. To reduce the chances of contamination of planting seed, a policy was adopted of ginning the planting seed first. Any contamination of planting seed would spread the disease widely, for planting seed of some companies is often bulked before distribution, and planted in fields scattered throughout the entire valley. Contamination would be detected only if the grower used sprinklers, soaked the seed before planting, or encountered heavy rains after seedling emergence.

Attempts were made to demonstrate the presence of the pathogen in gins that handled cotton from blighted fields. Samples of plant refuse and seed were taken primarily from augers of the inclined cleaner, gin stand, and feeder cleaner in several gins[3]. Since some growers use the refuse from the gin piles for fertilizer, samples were also taken from the gin piles.

A method of detection was devised that gives every advantage to the pathogen. Fifty-gram samples of the refuse and seed or refuse alone from the various portions of gins were soaked 18 hours in 500 cc of sterile distilled water. Fifty disease-free, acid-delinted Acala 4-42 seed were placed in containers with each sample. The refuse, seed, and suspensions were placed in large seedling pots in the greenhouse (80° F). Several weeks later, observations for the disease were made.

Angular leaf spot developed when healthy seed was soaked in water suspensions of cotton refuse or refuse and seed from augers in the inclined cleaner, and gin stand (Table 2). The disease did not develop when seed was soaked in suspensions of refuse taken from the gin refuse pile or the auger of the feeder cleaner (Table 2).

Primary infections of cotton in California have been traced to seedlings arising from infested seed. Since there is little rainfall in the San Joaquin Valley, decomposition of plant refuse

[3]The authors are indebted to Marvin Hoover, Extension Cotton Specialist, University of California, for his help in making these collections.

Table 2. Recovery of Xanthomonas malvacearum from refuse taken from various places in cotton gins in central California.

Source of refuse	Number of gins sampled	Number of gins from which pathogen was recovered
Auger inclined cleaner	3	2
Auger gin stand	3	2
Auger feeder cleaner	1	0
Gin refuse pile	1	0

is negligible, and debris probably constitutes an important reservoir of the pathogen. Under California conditions, the pathogen carries-over in virtually 100% of the fields with a history of the disease. Planting contaminated seed appears responsible for the occurrence of the disease in fields far-removed from those with high incidence of the disease, in fields that have been out of cotton production for many years, or on virgin soils. The pathogen is disseminated within a cotton field by sprinklers, and a field with only a few diseased plants can become heavily damaged as the summer progresses.

Use of acid-delinted seed has been recommended as a control measure for many years (2, 4, 5, 6, 14); tests in our laboratory have borne this out. Of 600 seed from severely infected bolls (by California standards), half were acid-delinted in concentrated sulfuric acid for 15 minutes and half were untreated. Both lots were then soaked 18 hours in sterile distilled water to enhance infection and were then planted in sterile soil. No angular leaf spot developed in the acid-delinted lot, whereas 10% of the plants from the untreated seed were infected. The results indicate little or no internal infection of cotton seed under California growing conditions, possibly because the boll-rot phase of the disease is usually not severe. The reason is probably low humidity and lack of rain before and during harvest.

DISCUSSION AND CONCLUSIONS

The major factor contributing to establishment of angular leaf spot of cotton in California appears to be sprinkler irrigation. The distribution pattern of the disease as of 1959 (Fig. 2) strongly suggests that infested seed is involved. Several fields of cotton showing angular leaf spot for the first time in 1958 were planted with seed from a single source. Since the pathogen may remain viable in cotton debris in cotton gins to infest new seed lots ginned in the following season, it is recommended that only gins handling seed from disease-free areas be allowed to process planting seed. Some contamination of planting seed by means of contaminated lint also could occur when the seed is mechanically delinted.

Most of the seed planted in the San Joaquin Valley is mechanically delinted. If irrigation is by sprinkler, planting seed should be acid-delinted and also should be treated with an organic mercury. Deep plowing plus the addition of moisture is recommended to bring about decomposition of infested debris and destruction of the pathogen.

Literature Cited

1. ARK, P. A. 1958. Longevity of Xanthomonas malvacearum in dried cotton plants. Plant Disease Reptr. 42: 1293.
2. BAIN, D. C. 1939. Effect of sulfuric acid treatment on fungi and bacteria present in cotton seed from diseased bolls. Phytopathology 29: 879-884.
3. BRINKERHOFF, L. A. 1959. Variability for pathogenicity of Xanthomonas malvacearum. (Abst.) Phytopathology 49: 543.
4. BROWN, J. G. 1942. Wind dissemination of angular leaf spot of cotton. Phytopathology 32: 81-90.
5. BROWN, J. G., and F. GIBSON. 1925. A machine for treating cotton seed with sulfuric acid. Arizona Agr. Exp. Sta. Bull. 105.
6. CHESTER, K. S. 1938. Gravity grading, a method of reducing seed-borne disease in cotton. Phytopathology 28: 745-749.
7. DICKSON, J. G. 1956. Diseases of field crops. McGraw-Hill Book Co., 2nd Ed. 517 pp.
8. DISTRIBUTION MAPS OF PLANT DISEASES. Revised 1952. Map No. 57, 2nd Ed. Commonwealth Mycological Institute, Kew, Surrey, England.
9. FAULWETTER, R. C. 1917. Dissemination of angular leaf spot of cotton. J.

Agr. Research 8: 457-475.

10. HARE, J. G., and C. J. KING. 1940. The winter carry-over of angular leaf-spot infection in Arizona cotton fields. Phytopathology 30: 679-684.
11. HUNTER, R. E., and L. M. BLANK. 1954. Pathogenicity differences of Xanthomonas malvacearum isolates. (Abst.) Phytopathology 44: 332.
12. MASSEY, R. E. 1934. Studies on black arm disease of cotton III. Empire Cotton Growing Rev. 11: 188-193.
13. REPORT OF THE NOMENCLATURE SUBCOMMITTEE. 1955. Proceedings of the 16th meeting of the Cotton Disease Council. p. 20.
14. ROLFS, F. M. 1915. Angular leaf spot of cotton. South Carolina Agr. Exp. Sta. Bull. 184.
15. ROLFS, F. M. 1935. Dissemination of the bacterial leaf spot organism. (Abst.) Phytopathology 25: 971.
16. TENNYSON, GERTRUDE. 1936. Invasion of cotton seed by Bacterium malvacearum. Phytopathology 26: 1083-1084.
17. WALKER, J. C. 1957. Plant Pathology. McGraw-Hill Book Co., 2nd Ed. 707 pp.
18. WEINDLING, R. 1948. Bacterial blight of cotton under conditions of artificial inoculation. U. S. Dept. Agr. Tech. Bull. 956. 59 pp.

DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF CALIFORNIA,
DAVIS, CALIFORNIA

LEAF SPOT OF PEANUT IN GEORGIA CAUSED BY LEPTOSPHAERULINA ARACHIDICOLA[1]

E. S. Luttrell and L. W. Boyle

Summary

The Ascomycete Leptosphaerulina arachidicola, described as the cause of leaf scorch of peanut in Taiwan in 1956, has been isolated from peanut leaves from six counties in Georgia. Results of inoculations indicate that this fungus is potentially capable of causing severe infection of peanut, producing symptoms ranging from leaf fleck to scorch.

In 1956 Yen, Chen, and Huang[2] published a description of a new disease of peanut in Taiwan under the name leaf scorch. They attributed this disease to an Ascomycete which they described as Leptosphaerulina arachidicola Yen, Chen, & Huang. A similar fungus was isolated from peanut leaves collected at Experiment, Georgia (No. 1325) in October 1958. In August 1959, the same fungus was obtained from peanut leaves from Baker (No. 1357), Stewart (No. 1344), Sumpter (No. 1343), Thomas (No. 1345), and Tift (No. 1356) counties in south Georgia. To determine their identity and pathogenicity the Georgia isolates were compared with a culture of L. arachidicola from Taiwan (No. 1337) sent by K. T. Huang, Provincial College of Agriculture, Taichung, Taiwan, on March 3, 1959.

SYMPTOMS

Symptoms as determined from artificially inoculated peanut seedlings (Fig. 1) varied from a leaf fleck or pepper spot to a leaf scorch resembling that described in Taiwan. Pepper spots were dark-brown lesions rarely 1 mm in diameter but usually smaller, ranging downward to

FIGURE 1. Pepper spot and leaf scorch symptoms produced on peanut leaves by Leptosphaerulina arachidicola. Upper three leaves from artificially inoculated seedlings. Lower two leaflets from naturally infected plants in Taiwan.

---

[1]Journal Series Paper No. 382, Georgia Experiment Station, Experiment, Georgia.

[2]Yen, J., M. J. Chen, and K. T. Huang. 1956. Leaf scorch of peanut (a new disease). J. Agr. Forestry (Taiwan) 10: 1-24. (Chinese; English summary).

tiny pin-point necrotic flecks. The lesions were sometimes circular but usually even the smallest spots were irregular in shape. When such spots were numerous, they formed a diffuse net blotch and resulted in death of the entire leaflet. On some leaves the lesions enlarged and became pale brown with dark brown irregular margins. Enlargement and coalescence of these spots produced irregular scorched areas in the centers or margins of the leaflets.

Symptoms observed in the field were confused by the common occurrence on the same leaves of Cercospora leaf spots in various stages of development. The common symptom appeared to be pepper spot. To confirm the diagnosis it was necessary to place the spotted leaves in a moist chamber until the characteristic ascocarps of the fungus developed or to make isolations from the lesions.

According to Yen, Chen, and Huang, the earliest symptoms of leaf scorch are yellow spots, usually produced along the leaf margin. When the lesion becomes 3 to 5 mm in diameter, the center changes from yellow to brown. The lesions gradually enlarge until the whole leaf blade is involved. The surface of old lesions is roughened by the numerous ascocarps of Leptosphaerulina arachidicola immersed in the dead tissue. Although this course of development has not been observed in Georgia material, the final appearance of the scorch lesions produced by inoculations with the Georgia isolates resembles that of the scorch lesions on peanut in Taiwan (Fig. 1).

THE FUNGUS

When spotted leaves were picked, allowed to wither and die, and placed on the surface of water or on moist filter paper in Petri dishes, the fungus spread rapidly throughout the leaf and produced mature ascocarps in less than a week. Ascospores were discharged upward onto the lid of the Petri dish in such numbers that they formed a print of the leaf. The fungus fruited in dishes in the laboratory exposed to diffuse light. In hot weather better development of ascospores was obtained in incubators with fluorescent lights at temperatures between 20° and 25° C.

The ascocarps were immersed, globose, 64-140 μ in diameter, with a membranous wall composed of pale-brown, polygonal, parenchymatous cells, and provided with a broad pore in a raised collar or short cylindrical neck of darker cells. Each ascocarp was filled with a few large, thick-walled, saccate, oval, or oblong asci measuring 53-87 x 28-42 μ. Most of the ascospores were muriform with 3-5 (usually 4) transverse septa and 2 longitudinal septa, ellipsoid, and constricted at the septa. From 16 to 36% of them were phragmosporous and cylindrical. Both types of spores occurred in the same ascus. The ascospores measured 23-40 x 10-17 μ and were hyaline when they were discharged from the asci. Old spores became pale to moderately dark brown. Leptosphaerulina arachidicola was originally described by Yen, Chen, and Huang as having spherical ascocarps 66-80 μ in diameter; ovoid asci measuring 56-72 x 30-32 μ; and oblong to ellipsoid, muriform, brown to olive-brown ascospores with 3-4 transverse septa and 1-2 longitudinal septa measuring 25-33 x 11-16 μ.

The six Georgia isolates and the Taiwan isolate were compared in culture on plates of 20% V-8 juice agar kept under fluorescent light in an incubator at 20° C. All produced similar colonies of dark, appressed mycelium densely covered with spherical black ascocarps. Observations on these isolates are recorded in Table 1.

Table 1. Morphology of Leptosphaerulina isolates in culture on 20% V-8 juice agar (Dimensions in microns).

Isolate	Ascocarp diameter	Asci length x width	Ascospores				
			Length x width	Mean	Number septa	Mean	% muriform
1325	104-196	64-84 x 34-45	27-35 x 11-15	32.5 x 13.5	3-5 x 0-3	4.4 x 1.7	84
1337	124-248	56-92 x 31-39	26-33 x 10-14	29.6 x 12.6	3-4 x 0-2	3.2 x 1.6	80
1343	101-174	59-90 x 34-42	27-37 x 11-15	32.4 x 12.9	3-5 x 0-2	4.0 x 1.4	72
1344	112-216	53-81 x 31-39	27-38 x 10-15	32.0 x 13.0	3-5 x 0-2	3.9 x 1.4	68
1345	106-182	62-90 x 31-36	29-37 x 11-15	32.9 x 13.0	3-5 x 0-2	4.0 x 1.4	68
1356	92-168	64-101 x 34-42	29-35 x 11-17	32.5 x 13.2	3-5 x 0.2	4.2 x 1.6	80
1357	90-174	59-90 x 36-42	27-38 x 11-15	32.2 x 12.8	3-5 x 0-2	3.7 x 1.5	76

INOCULATIONS

For pathogenicity tests, Spanish peanut seedlings were grown in 4-inch pots. Sporulating Petri dish cultures of the fungus were inverted over the seedlings on a wire frame and covered with a plastic freezer bag for 48 hours. In a series of inoculations in the greenhouse only pepper spot symptoms developed on the inoculated plants, although infection was severe enough to result in death and defoliation of many leaflets within a month. Inoculations made in growth chambers at 18° and 28° C resulted in more severe infections with symptoms of pepper spot and scorch. Flecking of leaves was apparent 2 or 3 days after the cultures were placed over the plants, symptoms of leaf scorch were well developed in less than a week, and considerable defoliation occurred by the end of the second week. Results of one series of inoculations with the six Georgia isolates and the Taiwan isolate are in Table 2. Differences in severity of infection shown among these isolates are of doubtful significance since greater variation occurred among plants inoculated with single isolates. On 16 plants inoculated with isolate No. 1356 infection varied from a trace of leaf flecking to 100% infection with 69% of the leaflets scorched and dead at the end of 2 weeks. A nearly identical range of infection was shown by eight plants inoculated with isolate No. 1337.

In inoculations made by inverting sporulating cultures over detached peanut leaves floated on water in Petri dishes typical symptoms failed to develop. On the detached leaflets all isolates produced mottled purplish areas which ultimately died and allowed the development of ascocarps.

Table 2. Results 2 weeks after inoculations with isolates of Leptosphaerulina on Spanish peanut seedlings in the four-leaf stage in a growth chamber at 18° C.

Isolate	Number of plants	Percentage of leaflets: infected	dead	scorch	pepper spot
1325	2	78	44	9	25
1337	2	72	25	0	47
1343	2	43	0	13	30
1344	2	75	6	3	66
1345	2	81	34	13	34
1356	2	37	6	6	25
1357	2	47	22	9	16
Control	2	0	0	0	0

CONCLUSIONS

Although the Georgia isolates tended to produce slightly larger ascospores with a greater number of transverse septa than the Taiwan isolate of Leptosphaerulina arachidicola, they were similar in their most striking morphological character, the production of two distinct types of ascospores. All isolates formed both ellipsoid, muriform ascospores and a smaller percentage of cylindrical, phragmosporous ascospores. As in the Georgia isolates, the ascospores of the Taiwan isolate were typically hyaline when they were ejaculated from the asci and became brown only with age. These isolates were similar also in their pathogenicity on peanut. Consequently, the Georgia isolates are referred to L. arachidicola.

Although Leptosphaerulina arachidicola was first found in Georgia in 1958, its common occurrence over the peanut belt here indicates that it has been present in this country for many years. It has probably escaped notice because the symptoms it produces are obscured and confused by those of the omnipresent Cercospora leaf spots. Inoculations demonstrate that L. arachidicola is potentially capable of causing severe infection on peanut. Additional observations are necessary to determine the symptomatology of the disease and its distribution and importance in the field.

GEORGIA EXPERIMENT STATION, EXPERIMENT, GEORGIA

DISTRIBUTION AND A MODE OF SPREAD OF CYTOSPORA CANKER IN AN ORCHARD OF THE PRESIDENT PLUM VARIETY IN CALIFORNIA

Luigi Chiarappa[1]

Summary

The incidence and distribution of Cytospora dieback in a mature President plum orchard revealed an average of 6.4 cankers per tree with the highest incidence on limbs exposed to the south. Young 4- to 5-year-old replant trees were found to be as severely diseased as older trees and the position of these in the orchard row apparently had no effect on disease occurrence. Cytospora sp. and few other organisms were isolated from active canker areas. Differences in pathogenicity were observed for various strains of Cytospora with only one strain appearing highly virulent. Different inoculation methods demonstrated that Cytospora readily invaded fresh cuts and that spore infested pruning tools were effective in spreading the disease.

Trees of the President plum variety (Prunus domestica) are known by California orchardists to be short lived. After reaching an age of 10 to 15 years these trees often "decline" rapidly and become severely affected by dieback. This condition, known to occur throughout the State, has recently been recognized to be caused by Cytospora spp., tentatively identified as C. rubescens Tul. and C. leucostoma Sacc. (8). Consistent association of C. rubescens with cankers of commercial species of fruit trees and some ornamentals affected by degeneration has been established in Utah (10). The existence of differences in virulence of this fungus has been observed (5) and demonstrated (11).

Symptoms of dieback in President plums similar to those described (5, 12) are most evident during the growing season when dying of entire branches or branch sections occurs. Leaves from diseased trees turn from normal green to a mottled yellow with green bandings bordering the main veins. They then dry out and remain attached to the limbs. Infected branches show typical collapsed bark areas coincident with the cankers. Often these are accompanied by abundant pockets which split open and release gum. Dark brown cankers are seen by removing the outer bark. They extend for various lengths and widths in the sapwood and are often preceded by streaks of necrotic tissues extending into areas otherwise appearing normal. Cankers which spread around the limb cause girdling and the resultant death of the distal portions.

Little is reported in the literature about epidemiology of the Cytospora canker disease. Lewis (7) regarded healthy branches as resistant to fungus attack, while others (8, 11) report dieback to be common also in otherwise healthy appearing trees. It is believed that infections are initiated in winter killed or weakened limbs (5, 10, 11). Cytospora spp. have been isolated from tissues surrounding tunnels of the shot-hole borer (Scolytus rugulosus Ratz.) (8), suggesting fungus spread by this insect. The oriental fruit moth (Grapholitha molesta Busck) and the peach tree borer (Sanninoidea exitiosa Say) are also reported to cause injuries in peach trees which later become cankered (12). Pruning wounds outnumber any other form of injury during the first 2 to 3 years of orchard history and serve as the main means of fungus entry (6, 12). However, small pruning wounds are probably seldom invaded by Cytospora because they heal over rapidly (1).

The purpose of this study was to determine the cause of dieback in three orchards of President plums and the distribution, incidence and mode of spread of the disease.

MATERIALS AND METHODS

Studies were conducted in three orchards of the President plum variety; the New England and Dantoni orchards, located near Marysville (Sacramento Valley), and an orchard near Arvin (lower San Joaquin Valley, California). Little dieback was observed in the Marysville orchards, while the Arvin orchard showed severe disease. The latter was selected for distribution and epidemiology studies of the disease and for pathogenicity tests. Ages of trees in the 24-acre Arvin orchard varied from 11 to 12 years.

Diseased branches on each of 1850 trees were recorded as "strikes" when one or more shoots were found dead. Strike counts were kept separate according to orientation of the limbs. The survey was made during the fall when most of the summer dieback was over and clinging

[1]Plant Pathologist, Di Giorgio Fruit Corporation, Di Giorgio, California.

dead leaves were a good visible symptom of the disease.

Isolations were made on potato-dextrose agar (PDA) adjusted at two different pH levels, 5.6 and 6.7, and all plates were incubated at room temperature. After purification of cultures by hyphal tip or by the single-spore method described by Hansen (4), the isolates were identified using the key by Barnett (2).

Pathogenicity tests were run in a manner essentially the same as that reported by Treshow et al. (11). Control inoculations consisted of thin plugs of sterile PDA. Limbs to be inoculated (1 to 2 cm in diameter) were selected on vigorous trees showing no disease symptoms. Inoculations were made in August and readings taken after 4 weeks.

Mode of infection and of fungus spread was studied in several ways. Direct penetration of Cytospora into uninjured limbs was tested by spraying spore suspensions on young shoots or large limbs which had been previously washed with water and detergent and then surface sterilized with 75% ethyl alcohol. One portion of each limb was left uncovered after spraying, while the other was wrapped in moist cotton and sealed with polyethylene. The cover was removed after 3 to 8 weeks. Spread through infested pruning tools was investigated by cutting healthy limbs with shears which had been flamed and subsequently either immersed in a fungus spore suspension or used to cut through active cankers. Pruning wounds made with sterile shears served as a control. Another method of inoculation consisted of spraying spore suspensions on fresh cuts of 1- or 2-year-old limbs. Some of these were immediately enclosed in moist cotton and polyethylene and the balance left uncovered. Sterile water applied to fresh cuts was the control. Inoculations were made during the middle of October and readings taken after 8 weeks.

RESULTS

1) Incidence and Disease Distribution: Dieback was found throughout the Arvin orchard (Table 1) with an average of 6.4 "strikes" per tree. The disease was more severe on the eastern side of the orchard where in previous years, according to the grower, a severe infestation of San Jose scale (Aspidiotus perniciosus Comst.) had considerably reduced the vigor of the trees. Disease occurrence decreased with distance from this area. On the other hand, limbs oriented to the south side had consistently more cankers than those in other positions on the tree. Except that the disease was more severe in the east side of the orchard, no differences in incidence of the disorder were found among trees in different positions in the orchard row. Age of the trees had no apparent effect on canker development. Young 4- to 5-year-old replants were as severely diseased as some of the older trees (Fig. 1).

2) Isolations: Several fungi were secured in culture by plating tissues from the margins of active cankers (Table 2). Some differences were observed in the type of isolates depending on the source of the diseased material. The Cytospora sp. from the Arvin area, identified as strain A, differed considerably from all other isolates and did not resemble the strains of C. rubescens described by Treshow and Scholes (10). The Arvin strain A, when grown on PDA, was at first white, then turned to a bright orange with some of the interspersed mycelium remaining white. Colonies had sparse mycelium and irregular margins. The medium surrounding older colonies became red to brown in color. Abundant pycnidia were produced in old fungus cultures. They were always imbedded in a felty, orange stroma and produced cirri of a dark amber color. Similar pycnidia were observed on diseased branches where they were completely hidden under the external layer of the outer bark. The other Cytospora isolates did not differ greatly from the description given by Treshow and Scholes (10). The unidentified organisms

Table 1. Incidence and distribution of branch dieback in a President plum orchard near Arvin, California.

Tree set number[a]	Number of strikes per quadrant				
	North	East	South	West	Total
1	164	218	266	165	813
2	161	292	322	221	996
3	446	566	644	508	2,164
4	530	539	654	465	2,188
5	793	802	926	750	3,261
Total	2,084	2,417	2,812	2,109	11,842

[a]Each tree set consisted of 10 rows of 37 trees each. Rows oriented north to south. Set numbers in progressive order from west to east.

FIGURE 1. Four-year-old President plum tree showing severe symptoms of Cytospora dieback. Arrows point to active cankers. Photo taken in mid January.

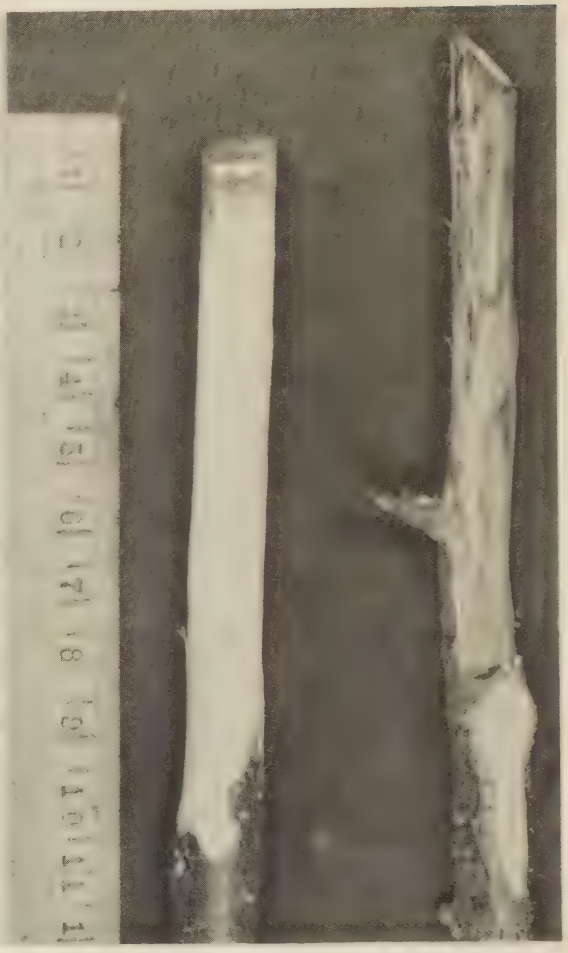

FIGURE 2. Canker formed from pruning wound. Results of inoculations made by the use of infested pruning shears after immersion in a Cytospora spore suspension. The outer bark has been removed to show discoloration of the tissues. Limb on the left was a control.

frequently obtained from Marysville and also present in the orchard near Arvin appeared to be wood inhabiting Basidiomycetes.

3) Pathogenicity Tests: Tissue discoloration and gummosis were produced in President plums by more than one organism (Table 3). However, typical canker formation was produced only where Cytospora strain A had been inoculated. Considerable differences in virulence among isolates of Cytospora were also evident as shown by the ability of strain B to produce some gummosis and tissue discoloration, while strain C had no effect on the host.

4) Methods of Disease Spread: Results of this phase of investigation (Table 4) revealed that a very efficient way of spreading the disease from one limb to another is by means of infested pruning tools. The heavy spore load obtained by immersing the blade of the pruning shears in a fungus spore suspension may account for the larger discolored area found in limbs treated by this method (Fig. 2). However, similar tissue discolorations were obtained where pruning shears were infested by previously cutting through active cankers. Where a spore suspension was sprayed on fresh wounds and these were immediately covered with polyethylene, a large number of pycnidia were produced throughout the cut surface. No pycnidia were present on limbs treated in the same manner but left uncovered. No gum formation was observed in any of the inoculated limbs.

Table 2. Source, identity and frequency of fungi isolated from cankerous limb areas of President plum trees.

Source	Fungi isolated	Number of cultures obtained	% frequency
Arvin	Cytospora, strain A	30	42.25
	Cytospora, strain B	1	2.50
	Pullularia pullulans	6	15.00
	Unidentified[a]	1	2.50
Dantoni, Marysville	Pullularia pullulans	2	8.33
	Unidentified[a]	12	54.14
New England, Marysville	Cytospora, strain C	1	4.17
	Pullularia pullulans	1	4.17
	Unidentified[a]	16	66.67

[a]Floccose, white, non-sporulating mycelium.

Table 3. Results of pathogenicity tests of various fungi isolated from President plum trees.

Isolate	Number of inoculations	Results
Cytospora, strain A	5	All positive cankers
Cytospora, strain B	3	Gummosis and tissue discoloration
Cytospora, strain C	2	Negative
Pullularia pullulans	3	Negative
Unidentified (Arvin)	4	Slight gummosis and limited tissue discoloration
Unidentified (New England, Marysville)	3	Negative
Control	8	Negative

Table 4. Effect of various inoculation methods on tissue invasion of President plums by a Cytospora isolate.

Inoculation method	Number of inoculations	Positive infections[a] (number)	Average length tissue discoloration (mm)
Spore suspension sprayed on uninjured limbs	6	0	--
Control	2	0	--
Spore suspension sprayed on fresh pruning wounds	6	5	25
Control	5	0	--
Pruning shears dipped in spore suspension	6	6	90
Control	3	0	--
Pruning shears contaminated by active cankers	4	4	20
Control	2	0	--

[a]Positive infections were those showing discoloration of tissues and yielding Cytospora by reisolation.

DISCUSSION

Cytospora canker was found to play a major role in the "decline" of a President plum orchard in California. Differences in virulence of various Cytospora isolates were in agreement with those reported for C. rubescens from Utah (11) and they may account for the delay in establishing the pathogenic role of this fungus.

The President plum variety was highly susceptible to a Cytospora isolate. Most likely sun scald injury has a great deal to do with its proneness to infection. President plum trees grow-

ing in the interior valleys of California are subject to sun cracks on exposed limbs and these openings may serve as avenues for penetration and establishment of the fungus. This hypothesis seems to find some support in that the highest incidence of cankers occurred on limbs oriented to the south. That the susceptibility of President plum may also be the result of varietal anatomical or physiological characteristics should also be investigated. In fact, anatomical differences in some Populus species recently have been shown to be correlated with resistance to Cytospora canker (3).

Wounds other than sun scald injuries may also allow limb penetration by Cytospora and other organisms. Infested pruning tools are demonstrated in this paper to be a highly efficient method of disease spread. The pattern of disease distribution in the Arvin orchard was suggestive of this possibility. The highest incidence of cankers in this orchard were concentrated in one area where it would seem that the disease first occurred. The number of cankers on surrounding trees decreased in almost a direct proportion to the distance from this area. Similar observations on successive reduction of cankers from a primary source have been reported in the literature (6).

On the basis of these studies it appears that it would be possible to lower the incidence and, possibly, the spread of Cytospora canker by reducing sun scald injuries (through cultural and chemical methods) and by careful disinfestation of pruning tools. A study to determine the effect of seasonal variation on susceptibility of pruning wounds, similar to that reported by Sharples (9), would be highly desirable to determine the most satisfactory time of pruning and the necessity of wound dressing materials.

Literature Cited

1. ANDERSON, H. W. 1956. Disease of fruit crops. McGraw-Hill. 501 pp.
2. BARNETT, H. L. 1955. Illustrated genera of imperfect fungi. Burgess Publ. Co., Minneapolis, Minnesota. 218 pp.
3. BLOOMBERG, W. J. 1959. Some anatomical evidence for resistance of Populus spp. to Cytospora canker disease. (Abst.) Rev. Appl. Mycol. 38: 490.
4. HANSEN, H. N. 1926. A simple method of obtaining single-spore cultures. Science (n.s.) 64: 384.
5. HELTON, A. W., and J. A. MOISEY. 1955. Cytospora damage in Idaho prune orchards. Plant Disease Reptr. 39: 931-943.
6. HILDEBRAND, E. M. 1947. Perennial peach canker and the canker complex in New York, with methods of control. Cornell Agr. Exp. Sta. Mem. 276.
7. LEWIS, C. E. 1912. Inoculation experiments with fungi associated with apple leaf spot and canker. Phytopathology 2: 49-62.
8. LUKEZIC, F. L., J. E. de VAY, and H. ENGLISH. 1960. Occurrence of Cytospora canker in stone fruit trees in California. (Abst.) Phytopathology 50: 84-85.
9. SHARPLES, R. O. 1958. The reduction of orchard sources of Gleosporium disease. Rep. Agr. Hort. Research Sta. Bristol, 1957: 105-114.
10. TRESHOW, M., and J. F. SCHOLES. 1957-1958. The taxonomy of some species of Cytospora found in Utah. Proc. Utah Acad. Sci., Arts and Ltrs. 35: 49-51.
11. TRESHOW, M., J. F. SCHOLES, and W. S. GARDNER. 1960. Pathogenic variation of Cytospora rubescens isolates on stone fruit varieties. (Abst.) Phytopathology 50: 86.
12. WILLISON, R. S. 1937. Peach canker investigations. III. Further notes on incidence, contributing factors and related phenomena. Can. J. Research 15: 324-339.

RESEARCH DEPARTMENT, DI GIORGIO FRUIT CORPORATION, DI GIORGIO, CALIFORNIA

ISOLATION OF OLPIDIUM BRASSICAE FROM ROOTS OF LETTUCE SHOWING BIG-VEIN SYMPTOMS[1]

Johanna T. Barr

Big-vein is a common and serious disease in Arizona-grown lettuce. The cause of the disease has been controversial but most recent studies indicate that Olpidium brassicae is responsible[2]. It has been possible to isolate this organism from head lettuce grown in soils from the Tucson, Yuma, and Mesa areas.

Roots were removed from infected plants and washed thoroughly in running tap water. After further washing in sterile distilled water, one of three methods was followed. Although all techniques reported provided growth, the first two proved unsatisfactory for further culture work.

1) Roots were placed in tubes of unsolidified water agar (12 g per liter) and slight growth appeared, but it was almost impossible to obtain distinct colony growth on streaked plates. This was due, in part, to the eventual dehydration of the agar which seemed to hinder further growth.

2) In hemp seed media (one hemp seed per 10 cc distilled water) a whitish filamentous growth appeared on the roots. Crowns of infected lettuce also showed this growth along with a reddening of the cortical area. Few zoospores formed. Transfers of loopfuls of this culture to other hemp media provided no growth at room temperature; nor did visible growth occur when transferred to tubes of water agar.

3) This method proved most satisfactory, as transfers could be made from one tube to another with rapid growth being produced in the second tube. Nutrient broth, when diluted, seemed to provide the most desirable medium for Olpidium at room temperature. Washed roots were placed in a dilution of two parts nutrient broth to one part sterile water. Within several days clouding of the medium developed. Growth consisted of numerous sporangia and some of the rudimentary filamentous growth. When loops of this solution were placed in a 1:1 dilution of the medium, zoospores and filaments appeared overnight. In undiluted broth (8 g Difco Nutrient Broth per liter distilled water) growth was less profuse.

A single spore isolating technique is being perfected. It is hoped that the success of this work will permit further pathogenicity studies of the organism.

ARIZONA AGRICULTURAL EXPERIMENT STATION, TUCSON

---

[1]Arizona Agricultural Experiment Station Technical Paper No. 595.

[2]Grogan, R. G., F. W. Zink, Wm. B. Hewitt, and K. A. Kimble. 1958. The association of Olpidium with the big-vein disease of lettuce. Phytopathology 48: 292-297.

BANANA FRUIT-SPOT CAUSED BY DEIGHTONIELLA TORULOSA (SYD.) ELL.

Donald S. Meredith[1]

Summary

Fruit-spot ("speckle") of Jamaican bananas is briefly described. Deightoniella torulosa (Syd.) Ell. was frequently isolated from spots. When pure cultures of the fungus were inoculated to healthy bananas in the field, typical fruit-spot developed after 7 days' incubation at a high humidity. Pathological histology showed that D. torulosa produces appressoria, from which an infection hypha grows out and penetrates the cuticle and epidermal cell wall. It was concluded that D. torulosa is the cause of this form of banana fruit-spot.

INTRODUCTION

Information on the subject of banana fruit-spots is scanty. However, in the Caribbean area, fruit-spotting may acquire considerable economic importance under certain conditions. In Jamaica a disease commonly known as "speckle" or "swamp-spot" is very severe at certain times of the year.

Wardlaw (12) reviewed the information on banana fruit-spot up to 1935. Most of the spots investigated by this worker (11) bear little resemblance to "speckle." In conclusion, he states that several fungi, for example Piricularia grisea, Helminthosporium torulosum, Gloeosporium musarum and Fusarium spp., may be associated with fruit-spots in a primary or secondary capacity. However, no experiments were conducted to determine which fungi act as causal agents.

The work of Johnston (4) and Johnston and Slocum (5), who investigated a spotting of Gros Michel bananas in Central America, is of particular interest. From their accounts it seems possible that they were studying the same form of fruit-spot that is described in this paper. The negative evidence that Johnston obtained from mycological and entomological studies led him to suggest that fruit-spot is a physiological disorder, and that spotting results from interruptions in the normal transpiration of the fruit and possibly from a consequent accumulation of toxic substances. Low evaporation is accentuated by heavy rainfall and high humidity. Johnston (4) also found that severe spotting is associated with low nitrate content of the soil.

In this paper, evidence is presented which suggests that fruit-spot of Jamaican Lacatan bananas is caused by the fungus Deightoniella torulosa (Syd.) Ell.

THE FUNGUS

D. torulosa (syn. Brachysporium torulosum, Syd., Cercospora musarum Ashby, Helminthosporium torulosum (Syd.) Ashby) is common in most banana-growing countries of the world. It has been recorded as causing a "black-spot" disease of banana leaves (1, 9) and a finger-tip infection ("black-tip") of fruits (8, 10). In addition, it may cause a stem-rot of the Manila hemp plant (2). The fungus also grows saprophytically on dead or dying banana tissues. Wardlaw (11) isolated the fungus from some forms of fruit-spot, but he did not test the pathogenicity of his isolates. Recently, Ellis (3) presented reasons for including the fungus in the genus Deightoniella.

FRUIT-SPOT SYMPTOMS IN JAMAICA

Fruit-spot on Lacatan bananas in the field is round, or nearly so, with a reddish-brown or black center and a darker green, apparently water-soaked halo. It may be depressed slightly below the level of surrounding healthy epidermis. Spots may be observed on fruit that has just "shot" and they become more numerous as maturity advances. They are most frequent on the insides of the fingers and also towards the finger-tips on all sides (Fig. 1). Histological details of spots are similar to those already described by Johnston (4) and Johnston and Slocum (5).

[1]Plant Pathologist, Banana Board Research Department, Kingston, Jamaica, W.I.

ISOLATION OF DEIGHTONIELLA TORULOSA

Wind-blown bunches that had rested on the plantation floor often developed severe spotting on the uppermost fingers; this was particularly true during rainy weather. In contrast, the fingers underneath were only slightly affected. Such a distribution of spots strongly suggested a downward movement of infective units impacting on the exposed fingers. One such stem was brought to the laboratory and, after thorough surface sterilization, spotted tissue was transferred aseptically to potato-dextrose agar plates. After 8 days' incubation of the agar plates at 28°C, D. torulosa had grown out of 90% of the excised spots; no other fungus appeared on the isolation plates. This high frequency of isolation suggested a causal relation between D. torulosa and fruit-spot. Many similar isolations from bunches hanging from the plant also yielded D. torulosa.

PATHOGENICITY OF DEIGHTONIELLA TORULOSA

Inoculations were carried out in the field by spraying fruit of different ages with a dense suspension of D. torulosa spores, obtained from 15-day-old agar cultures. After inoculation, the bunches were covered with air-tight plastic bags in order to maintain a high humidity at the fruit surface. Control bunches were not inoculated but were similarly bagged. When the experiment was re-examined after 7 days, inoculated fruit was found to be severely spotted; controls were relatively free from spotting.

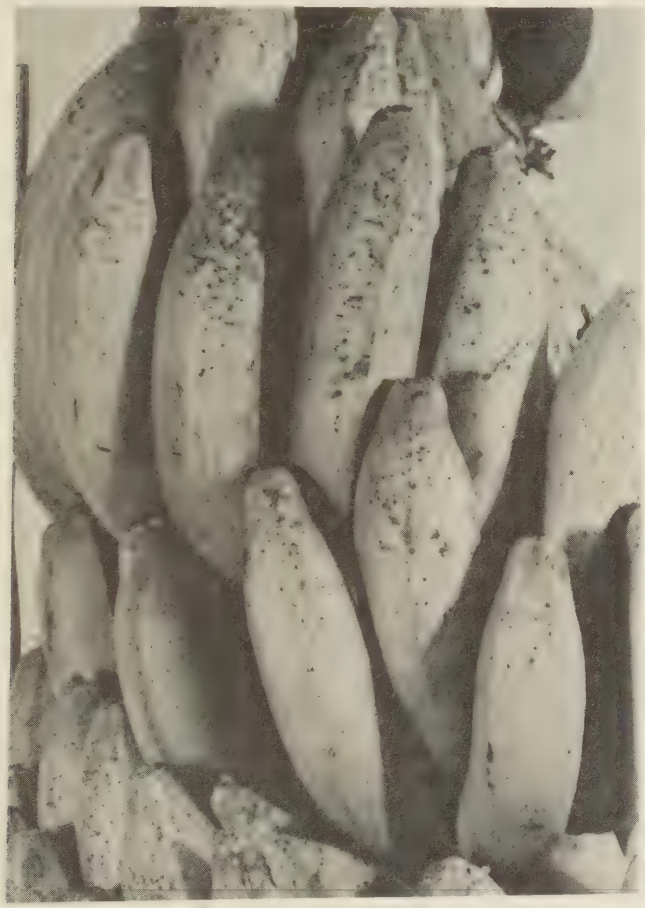

FIGURE 1. Symptoms of fruit-spot on banana.

Other inoculations were carried out in the laboratory. Small segments of healthy, spot-free peel cut from immature green bananas were sprayed with a suspension of conidia of D. torulosa and incubated at 28° to 30°C inside damp chambers. At the end of a week many spots appeared on inoculated segments, but none developed on un-inoculated controls. The experimentally induced spots were identical, both macro- and microscopically, with naturally occurring ones.

Final proof of pathogenicity of D. torulosa was obtained from histological studies. After germination on the surface of the banana fruit, germ tubes produce appressoria (Fig. 2). The appressorium is firmly attached to the epidermal layer of cells and produces a relatively fine infection hypha which penetrates the cuticle and epidermal cell wall. Penetration may occur after 6 hours, but in the majority of cases takes 12 to 20 hours. Soon after penetration has occurred the invaded epidermal cell assumes a reddish-brown color, due to the presence of gummy or tannin-like substances; the cell wall also becomes discolored. Further tissue penetration then occurs, usually by means of hyphal penetration of cell walls (Fig. 3).

All stages of infection were observed on naturally infected fruit. On the smallest spots, hand surface sections often revealed collapsed conidia of D. torulosa attached to germ tubes and appressoria. The expended conidia are readily identified by the persistence of the black annulus at the base (conidiophore end) of the conidium (Fig. 4).

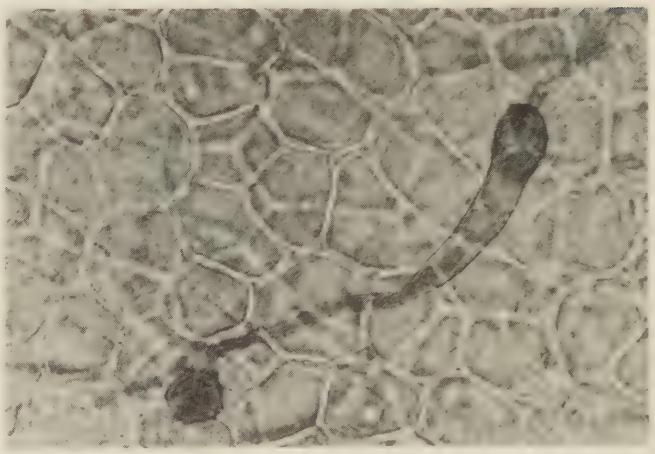

FIGURE 2. Germinated conidium of D. torulosa on surface of banana fruit epidermis. The circular appressorium has developed in the grooves between adjacent epidermal cells.

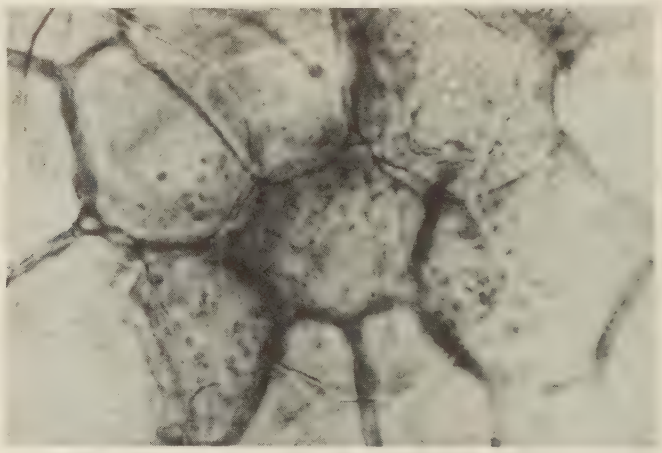

FIGURE 3. Section of fruit-spot tissue showing intracellular mycelium of D. torulosa.

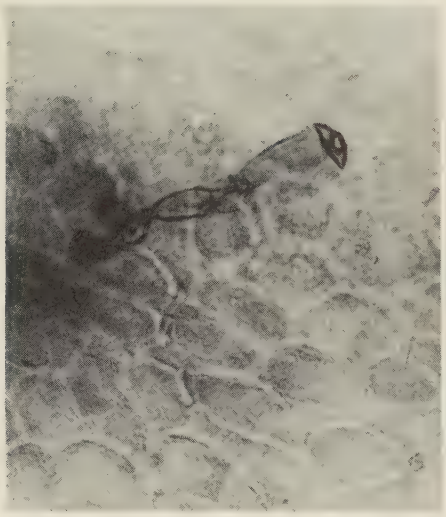

FIGURE 4. Early stage in natural infection of banana fruit by D. torulosa. Cells immediately below lobed appressorium are necrosed. Note the black ring at the base of the conidium -- a useful aid to identification of germinated conidia.

DISCUSSION

The observations made suggest that D. torulosa is the cause of fruit-spot ("speckle", "swamp-spot") of Jamaican Lacatan bananas. Until suitable material becomes available for comparison, it is not possible to ascertain whether the fruit-spot described by Johnston (4) is identical with "speckle." It would be of interest to discover whether inoculation of Gros Michel bananas in other Central American countries results in the development of similar spots. Observations by Meredith (6, 7) show that symptom expression in fruit infected by D. torulosa varies according to age of fruit and climatic and soil conditions.

The most important factor affecting the incidence of fruit-spot appears to be humidity. The writer (7) has found that germination of D. torulosa does not occur below 95% R.H. and is highest in a film of water. Disease is most severe following heavy rainfall.

It is of interest that details of penetration of banana fruit are similar to those described by Stahel (9) for "black-spot" of leaves. On leaves, the lesions caused by this fungus are often extensive, in contrast to the relatively small spots on fruit. Possibly physiological conditions in fruit-peel tissue limit extensive invasion by the fungus.

On the basis of what is now known about the etiology of Deightoniella fruit-spot, experiments have been set up to attempt to formulate economic control measures.

Literature Cited

1. ASHBY, S. F. 1913. Banana diseases in Jamaica. Bull. Dept. Agr., Jamaica 25: 95-128.
2. CALINISAN, M. R., J. A. AGATI, and V. C. ALDABA. 1931. Preliminary notes on the stem-rot of Abaca in the Philippines. Philipp. J. Agr. 2: 223-227.
3. ELLIS, M. B. 1957. Some species of Deightoniella. Mycol. Pap. Commonwealth Mycol. Inst. No. 66. 12 pp.
4. JOHNSTON, J. R. 1932. The fruit spot of the banana. Res. Dept., United Fruit Co., Boston, Mass. Bull. No. 43, 69 pp.
5. JOHNSTON, J. R., and A. H. SLOCUM. 1932. The banana fruit spot and its control. Res. Dept. United Fruit Co., Boston, Mass. Bull. No. 41, 14 pp.
6. MEREDITH, D. S. Fruit-spot ("speckle") of Jamaican bananas caused by Deightoniella torulosa (Syd.) Ell. I. Symptoms of disease and studies on pathogenicity. Trans. Brit. Mycol. Soc. (In Press).
7. MEREDITH, D. S. Fruit-spot ("speckle") of Jamaican bananas caused by Deightoniella torulosa (Syd.) Ell. II. Factors affecting germination and infection. Trans. Brit. Mycol. Soc. (In Press).
8. OGILVIE, LAWRENCE. 1928. "Black tip," a finger-tip disease of the Chinese banana in Bermuda. Phytopathology 18: 531-538.
9. STAHEL, G. 1934. The banana leaf disease in Surinam. Trop. Agriculture, Trin. 11: 138-142.
10. WARDLAW, C. W. 1932. Banana diseases. 4. Notes on "black-tip" disease in Trinidad: Helminthosporium torulosum (Syd.) comb. nov. Ashby. Trop. Agriculture, Trin. 9: 3-6.
11. WARDLAW, C. W. 1934. Banana diseases. 6. The nature and occurrence of pitting disease and fruit spots. Trop. Agriculture, Trin. 11: 8-13.
12. WARDLAW, C. W. 1935. Diseases of the banana. Macmillan Company, London, 615 pp.

BANANA BOARD RESEARCH DEPARTMENT, KINGSTON, JAMAICA, W.I.

AN ASSAY FOR THE DETECTION OF NEMATODE REPELLENTS[1]

D. Davis and J. E. Deak

Abstract

Panagrellus redivivus preferentially collects on colonies of Fusarium oxysporum f. lycopersici. Based on this observation, an assay was developed to detect chemicals repellent to nematodes. Such factors as temperature, substrate, optimum age of Panagrellus, number of nematodes per dish, number of Fusarium colonies per dish, were investigated to determine the conditions for maximum efficacy of the assay. A number of conclusions concerning practical utilization of a repellent, its advantages and application in other areas of pest control are discussed. The implications of the Fusarium-Panagrellus interaction are discussed in relation to the fungus-nematode complex in root disease.

The chemicals currently used to control plant parasitic nematodes are either nematistatic or nematicidal. Since nematodes are mobile animals possessing the sensory perceptions of taste and touch, it is reasonable to assume that they can also respond to chemical repellents. However, lacking a method of detecting repellents, this area of nematode control has been unexplored. The observation that Panagrellus redivivus (Linn.) Goodey preferentially collects in areas of a Petri dish harboring colonies of Fusarium oxysporum f. lycopersici has made possible development of an assay for the detection of chemical repellents.

EXPERIMENTAL

When a mixture of larvae and adults of P. redivivus is placed in a Petri dish supporting young colonies of various fungi, the nematodes become most heavily concentrated on F. oxysporum f. lycopersici. They are considerably less numerous on colonies of Pythium debaryanum, Rhizoctonia solani, Aspergillus sp. and Penicillium sp. and almost absent from colonies of Alternaria solani. Consequently, F. oxysporum f. lycopersici was used as the "attractant" throughout this investigation. Numerous observations on the movement of a fixed number of nematodes from the center of a Petri dish demonstrated that accumulation of Panagrellus on the Fusarium colony is not a chemotaxic response. Two 48-hour-old Fusarium colonies were located at opposite ends of the dish. The nematodes arrived with the same frequency at the quadrants devoid of the Fusarium colonies as they did at quadrants containing them. However, once the nematodes contacted the colony, they moved out of its environs with less frequency than from other areas of the Petri dish, resulting in a non-random distribution of nematodes on the PDA surface. This does not preclude the possibility that a diffusible substance produced by F. oxysporum f. lycopersici is responsible for the accumulation of P. redivivus around the Fusarium colony. It merely indicates that a sufficiently high concentration of the "attractant" does not diffuse for a considerable distance beyond the Fusarium colony. Lownsbery and Viglierchio (1) designed an ingenious device which answered this question in relation to the accumulation of Meloidogyne incognita acrita on tomato seedlings. They found that accumulation of the nematodes was in part due to a dialyzable substance emanating from the seedling.

Larvae and adult nematodes used in this study were obtained by growing Panagrellus in a non-sterile "Quick Mother's Oats"oatmeal made up to a pasty consistency with distilled water. Two weeks after the oatmeal was inoculated with Panagrellus, a mixture of larvae and adults were scraped from the oatmeal-free sides of the glass container. The nematodes were then washed four to five times with distilled water by decantation, after centrifugation with a small hand-driven centrifuge or by allowing the nematodes to settle at the bottom of a test tube. This procedure was necessary to avoid heavy contamination of the Petri dish with yeast brought in on the bodies of Panagrellus.

The Fusarium used in this study was grown in Petri dishes on PDA. When the culture was 1-week old, a 1/16 inch plug, made with a cork borer, was removed and transferred to four quadrants of a Petri dish.

Results from one of the earliest experiments clearly demonstrated that a nutrient agar

---

[1]We wish to thank Mr. Arthur Itkin for the statistical analysis.

matrix, in contrast to water agar, was necessary for the accumulation of the nematodes at the Fusarium plug. This was correlated with the lack of fungus growth beyond the Fusarium plug in the absence of nutrients. Thus 25 times as many nematodes were found on 24-hour-old Fusarium colonies growing on PDA than on water agar. Accordingly, PDA was used as the supporting matrix in all the other experiments described in this paper.

The results of another experiment demonstrated that the attraction of Panagrellus to the Fusarium plug was independent of the age of the oatmeal culture. The nematodes derived from a 2-week-old culture accumulated at the Fusarium plug was the same frequency as those from a 3-, 4-, or 5-week-old culture.

The optimum age of the Fusarium colony for maximum nematode "attraction" was also investigated. Results showed that a majority of nematodes accumulate at the Fusarium colony whether it is 8, 20 or 40 hours old at the time the nematodes are added to the dish, provided the count is made 24 hours after the nematodes are placed in the dish. Since this information was obtained from three separate experiments, each run at one of the above-mentioned time intervals, it was not valid to pool the data for an analysis of variance. Nonetheless, it is apparent that either 8-, 20-, or 40-hour-old colonies would be satisfactory for the repellency assay. Still another experiment demonstrated that significantly more nematodes were found at the Fusarium colony when the count was made 24 rather than 8 hours after Panagrellus was placed in the dish ($P < 0.01$). An average of 206 nematodes were found at each plug in 24 hours in contrast to 155 in 8 hours. In this experiment approximately 1000 washed nematodes were placed in the center of a Petri dish with four 24-hour-old Fusarium colonies in each quadrant.

The interaction of three variables was investigated in a final experiment. The data in Table 1 summarize the results. The following conclusions were reached based on an analysis of variance. (a) There is no significant difference in the relative number of nematodes which accumulate around a Fusarium colony whether 100 or 1000 nematodes are added to the center of the Petri dish. (b) A significantly higher percentage of nematodes are concentrated around the Fusarium colonies when four rather than one colony is used per dish ($P < 0.05$). (c) Significantly more nematodes accumulate at the colonies at 70° F than at 85° ($P < 0.01$). Furthermore, yeast contamination is excessive at 85° even if the nematodes are washed. (d) None of the interactions between the two variables significantly alters the first three conclusions.

Table 1. The effect of temperature, number of nematodes per dish, and number of Fusarium colonies per dish on the "attraction" of P. redivivus to F. oxysporum f. lycopersici.

Incubation temperature ° F	Number of nematodes/dish	Number of Fusarium colonies/dish	Number of nematodes at Fusarium colony				
			1	2	3	4	Average
70	100	1	75				75
70	100	4	23	23	23	28	24
70	1000	1	766				766
70	1000	4	220	233	233	208	223
85	100	1	67				67
85	100	4	23	22	20	27	22
85	1000	1	617				617
85	1000	4	200	217	167	200	196

REPELLENCY ASSAY PROCEDURE

Based on the previously described data, the following assay was developed for the detection of chemical repellents and/or attractants. Fifteen ml of sterile Difco PDA (39 g/1000 ml of distilled water) is poured into a Petri dish (9 cm) and allowed to solidify. Four 12.7 mm diameter filter paper discs (No. 740-E, Carl Schleicher and Schuell Co.) treated with the test chemical are then placed at each quadrant and near the outer edge of the dish. Finally, 15 ml of sterile PDA is poured gently into the Petri dish so as not to disturb the paper discs. The chemical is applied to the paper disc by dipping the disc in a known concentration of chemical solution or suspension and shaking off the excess solvent, or by pipetting a fixed quantity of solution on the paper disc. 0.1 Ml. will just saturate the paper discs. The paper disc is dried at room temperature before it is placed on the PDA. After the upper layer of PDA has solidified, a 1/16-inch Fusarium plug, taken from a week-old culture grown on PDA in a Petri dish, is placed on the PDA directly over each paper disc. Twenty-four hours later, one drop of a nematode suspension which has been washed four to five times with distilled water is placed in the

center of the dish. Each drop contains a mixture of approximately 1000 larvae and adult nematodes derived from a 2-week-old oatmeal culture of P. redivivus. Twenty-four hours after the nematodes are added, a count is made of the nematodes at each Fusarium colony.

If a compound is repelling the nematodes, there should be fewer nematodes at the fungus colony than expected. Conversely, if more nematodes are found at a Fusarium colony, the compound is acting as an attractant. In the initial assay, the paper discs at each quadrant contained a different test compound. When a compound was found which appeared to repel or attract the nematodes, it was retested by placing two paper discs treated with the same compound in opposite quadrants and two untreated paper discs in the other two quadrants. This diminished the chances of a distorted count caused by interference from the other compounds in the Petri dish. It, of course, also served as a repeat trial.

Compounds with antifungal activity will confound the results of the assay. As might be expected, when a test chemical retards Fusarium growth, fewer nematodes are found at the Fusarium plug. Accordingly, whenever a compound appears to repel Panagrellus, the growth of the Fusarium colony is checked closely to determine whether the absence of nematodes is due to a lack of fungus growth or the direct effect of the test compound.

Although a large number of compounds were tested by the method just described, none was found which consistently repelled or attracted P. redivivus. Accordingly, a typical response in the absence of an active material could be represented by that portion of the data in Table 1 in which the temperature is maintained at 70° F and 1000 nematodes placed in a dish containing four Fusarium colonies.

DISCUSSION

A nematode repellent would be practical for application either to (a) the soil in the planting row (conversely, an attractant would have to be placed in the furrow) or (b) to the foliage if it possessed systemic activity and could be translocated to the roots.

Since phytotoxicity is a major consideration in chemical nematode control, the search for repellents may prove more rewarding than the search for nematicides. It is a reasonable probability that a compound with repellent activity is less likely to be phytotoxic than a nematicide. This assumption is based on the probable absence of reactive sites, responsible for sensory perception phenomena, in higher plants analogous to those in animals. Nematicides, on the other hand, are more likely to be general protoplasmic inhibitors which interact on the molecular level with cell components common to both nematodes and higher plants.

Since the repellency assay is relatively inexpensive, rapid, and easy to perform, it might also be used as a primary screen for the detection of chemicals repellent to other animal pests such as insects and vertebrates.

The implications of this investigation extend beyond the repellency assay. Many root diseases are caused by fungus or bacteria-nematode complexes. The relationship between these organisms in pathogenicity is poorly understood. For example, the incidence and severity of Fusarium wilt of cotton is commonly believed to be increased by plant parasitic nematodes. The nematodes presumably increase susceptibility by producing wounds through which the pathogen can penetrate the root. Wilhelm (2) cites evidence which casts doubt on this explanation. Does the Fusarium-Panagrellus interaction suggest that wilt severity in cotton is increased by the accumulation of an injurious nematode population at sites of Fusarium concentration in the root? This and other questions concerning the root-disease complex may be clarified by the observation reported in this paper.

Literature Cited

1. LOWNSBERY, B. F., and D. R. VIGLIERCHIO. 1960. Mechanism of accumulation of Meloidogyne incognita acrita around tomato seedlings. Phytopathology 50: 178-179.
2. WILHELM, S. 1959. Parasitism and pathogenesis of root-disease fungi. Plant Pathology, Problems and Progress, 1908-1958. The University of Wisconsin Press, Madison, Wisconsin.

MERCK SHARP & DOHME RESEARCH LABORATORIES, RAHWAY, NEW JERSEY

SOME SAP-STAIN FUNGI FOUND IN MINNESOTA[1]

Robert N. Campbell[2]

Summary

Fungi isolated from sapwood of logs, lumber, or stumps of native tree species in Minnesota were: Ceratocystis fimbriata from Quercus ellipsoidalis; C. moniliformis from Populus tremuloides; C. pluriannulata from P. tremuloides and Q. ellipsoidalis; C. piceae from Populus sp.; C. pilifera from Pinus banksiana, P. resinosa, P. strobus, and Picea sp.; C. coerulescens and C. ips from Pinus banksiana and P. resinosa. Leptographium sp. was commonly isolated from P. strobus and once each from the other pines and spruce. Graphium spp. were isolated from the three pines and Q. ellipsoidalis. This is the first time that these species of Ceratocystis, except C. ips, have been reported from Minnesota. With the exception of C. pilifera on Pinus strobus, C. moniliformis on Populus tremuloides, and C. ips on Pinus resinosa and P. banksiana, the hosts reported herein are new substrates for the species named.

Fungi that cause sap-stain have been studied in most parts of the country, such as the Southern region (3, 4, 9, 17, 18), the Rocky Mountain and Pacific regions (5, 6, 7, 15, 16, 19, 20), and the Eastern region (13, 14, 21). The only stain fungi previously reported from Minnesota are Ceratostomella ips Rumbold (11, 15), and Tuberculariella ips Leach, Orr & C. Christensen (11) on red pine. Leach et al. (11) described the symbiotic association of these two fungi and two bark beetles, Ips pini Say and I. grandicollis Eich. The present paper reports on the wood-staining fungi collected in Minnesota during the summers of 1955 and 1956. Species of Ceratocystis were studied most intensively, but species of the form genera Graphium and Leptographium were collected also.

The monograph by Hunt (10) has been used here as the basis for identification of the Minnesota isolates.

MATERIALS AND METHODS

The fungi were isolated by aseptically removing chips of stained wood and placing them on malt agar or by putting infected samples of wood in a moist chamber and transferring exuded ascospores or conidia.

Pure cultures of the fungi were maintained on 2% malt agar. These fungi have a tendency to change from the perithecial to non-perithecial type in culture. However, by transferring only ascospore masses, vigorous, perithecium-producing cultures have been maintained for over 4 years.

The fungi were examined and described in pure culture since two or three species commonly were present on a given sample of wood. The fungi were mounted in lactophenol or lactofuchsin (2). The latter stain was found to be especially useful in staining endoconidiophores and endoconidia. Only significant differences from the descriptions (10) are presented here.

RESULTS

The species isolated from various hosts are summarized in Table 1. These isolations were made from lumber or slabs with the following exceptions: C. ips was collected from cut trees which had been attacked by Ips beetles, and the isolations from Quercus ellipsoidalis were made only from stumps of recently cut trees. The following summarizes the observations on the amount of stain caused by the species and how they differed morphologically from published descriptions.

---

[1]Paper No. 4348, Scientific Journal Series, Minnesota Agricultural Experiment Station.

The author is indebted to Dr. E. E. Butler for thoughtful criticism of the manuscript, and to Dr. D. W. French for assistance during the course of the study.

Mr. Ross W. Davidson and the late Dr. John Hunt kindly verified the identification of some of the isolates and provided cultures for comparison.

[2]Former Research Fellow, Department of Plant Pathology and Botany, University of Minnesota; now Assistant Plant Pathologist, University of California, Davis.

Table 1. The sap-stain fungi isolated or identified from stumps or lumber in Minnesota.

Host	Fungi	Number of times isolated or identified
Pinus resinosa (red pine)	C. pilifera (Fries) Moreau[a]	9
	C. coerulescens (Münch) Bakshi[a]	4
	C. ips (Rumbold) Moreau	3
	Leptographium sp.	1
	Graphium sp.	2
P. banksiana (Jack pine)	C. pilifera[a]	5
	C. coerulescens[a]	3
	C. ips	3
	Graphium spp.	3
	Leptographium sp.	1
P. strobus (white pine)	Leptographium spp.	4
	C. pilifera[b]	3
	Graphium sp.	3
Picea sp. (spruce)	C. pilifera[a]	2
	Leptographium sp.	1
Populus tremuloides (aspen)	C. pluriannulata (Hedgc.) Moreau[a]	5
	C. moniliformis (Hedgc.) Moreau[b]	5
	C. piceae (Münch) Bakshi[a]	3
Quercus ellipsoidalis (northern pin oak)	C. pluriannulata[a]	3
	C. fimbriata Ell. & Halst.[a]	2
	Graphium sp.	1

[a] New host record and new record for Minnesota.

[b] New record for Minnesota.

C. fimbriata appeared as limited grey patches on the wood surface and there was no stain of the underlying wood. In a previous paper (1) this isolate was reported as C. variospora (David.) Moreau, although it was known that it did not fit clearly into either C. variospora or C. fimbriata because of conidial differences. C. fimbriata has two endoconidial spore forms: hyaline, cylindrical endoconidia borne in chains, and olive-brown, subglobose to pyriform endoconidia (or macroconidia) borne in chains. C. variospora has a hyaline, cylindrical endoconidial stage, a hyaline, barrel-shaped endoconidial stage, and an olive-brown, exogenous macroconidium borne singly on a branched conidiophore. The Minnesota isolate possessed the cylindrical, hyaline endoconidia common to both species, the barrel-shaped, hyaline endoconidia reported for C. variospora, and olive-brown, globose to pyriform endoconidia identical to those reported for C. fimbriata. There are no differences in perithecial morphology sufficient to distinguish between the two species in this author's opinion. Since there is in the literature a report of C. fimbriata (as Endoconidiophora fimbriata (Ell. & Halst.) Davidson) possessing the barrel-shaped endoconidia (3), the present isolate has been determined to be C. fimbriata. The barrel-shaped, hyaline endoconidia measured 6-9 x 4.5-6μ and were borne in brown, short endoconidiophores 27-75μ long and flaring from 4-4.5μ at base to 4.5μ at the tip. These are clearly distinct from the hyaline, cylindrical endoconidia which were 18-39 x 3-4μ and borne in septate, pale brown endoconidiophores 80-140μ long, tapering from 6-7μ diameter near the base to 3-4μ at the tip. The olive-brown, globose to pyriform endoconidia were as described for C. fimbriata (10).

C. coerulescens formed a deep blue-black layer of mycelium and endoconidiophores on the surface of infected wood. Within the wood it caused a deep blue stain. This was the typical softwood strain of the fungus, not the hardwood strain that was described as Endoconidiophora virescens Davidson (4).

C. moniliformis was associated with a brown surface discoloration in aspen and a very light blue stain in the wood. The isolate studied produced cylindrical endoconidia and moniliform conidia, not borne endogenously, which became brown with age and measured 7-11 x-14μ on conidiophores 9-21 x 3-5μ. The latter spore form is previously unreported for this species. These conidia were produced abundantly and were formed basipetally by rounding up of mycelial cells. The barrel-shaped endoconidia were not formed by this isolate. Since the present

isolate agrees in details of the sexual stage it is retained here in C. moniliformis.

The incubation temperature was important in determining the colony color and amount of conidial sporulation by C. moniliformis. Cultures incubated 4 days at 15°, 20°, and 25°C were white. In the colonies grown at 25° there were many endoconidia but few conidia. Plates incubated at 30° had light brown colonies bearing many endoconidia and abundant conidia. Apparently at 30° the formation of conidia of both types occurred most profusely, and the brown coloring material of the conidia and of the mycelial cell walls was formed more abundantly.

C. ips did not produce the Graphium-like conidial structures reported by Hunt (10). Leach et al. (11) have excellent illustrations of the stain caused by the fungus.

C. piceae caused only a very light stain in the wood of aspen. The perithecia were 160-250μ in diameter (mean 185μ) with black necks 1400-1800μ long (mean 1625μ) compared with 80-180μ diameter with necks up to 1000μ long (10).

C. pluriannulata caused a light blue stain in the wood in aspen and northern red oak.

C. pilifera, the fungus most frequently isolated from coniferous wood, caused a light to dark blue stain. It was easily recognized by the numerous perithecia borne on the surface of the infected wood.

DISCUSSION

Although this study was limited in extent, it is interesting that the same species of Ceratocystis (Ceratostomella) found to be the most serious hardwood and softwood stain fungi in the southern United States (18) were among those most frequently encountered in Minnesota.

Throughout this study it was possible to isolate the endoconidial species only when the wood was still fresh and moist. After the wood had dried out, none of these fungi was isolated, although the perithecia and endoconidiophores were present. However, C. pilifera on pine lumber or C. pluriannulata on aspen boards were isolated frequently from such pieces. Apparently the endoconidial group is much less resistant to desiccation than are other species of the genus.

The Minnesota isolates of C. fimbriata and C. moniliformis both differed in conidial stages from the generally accepted descriptions. These findings, and the recent findings (8) that in Ceratostomella radicicola Bliss the production of the macroconidia was governed by a single gene which could mutate resulting in the lack of these spores, emphasize that perithecial morphology is the primary basis for speciation and that variation, at least in those species with endoconidia, in the conidial stages formed by an individual isolate is to be expected. This is supported by the previous report of C. fimbriata possessing the barrel-shaped endoconidia (3) and the finding of a Graphium stage for Ceratocystis major (van Beyma) Moreau (12).

In regard to C. fimbriata and C. variospora the question should be raised as to whether they are separate species. The differences in perithecial morphology between them is indeed small (10): the ostiolar hyphae of C. variospora are shorter (10-20 ostiolar hyphae, 20-35 x 1-2μ compared with 8-15 ostiolar hyphae, 50-90 x 2-3μ); the ascospores are smaller (4-6 x 2.5-3.5μ compared with 4.5-8 x 2.5-5.5μ); and the perithecial bases are light colored ("light brown to black," Davidson) compared with brown to black for C. fimbriata. In addition, the Minnesota isolate was intermediate between these descriptions, with 8-26 ostiolar hyphae 21-56μ long, perithecial bases black, and ascospores 4.7-5.4 x 2.7μ. Since C. variospora has been studied from the type collection and not from cultures -- as has C. fimbriata -- it is questioned whether the differences between the species are not due to the environment under which the fungi developed. The only remaining differences between the species are in the olive-brown macroconidia which were reported to be borne exogenously and singly on alternately branched conidiophores in C. variospora and endogenously in chains in C. fimbriata. Again, it is not known to what extent this spore form is affected by cultural conditions or the expected variability between isolates. One of the olive-brown macroconidia figured in the original C. variospora description has the pyriform shape that is typical of the olive-brown conidia produced endogenously in C. fimbriata. Final answers to this question will necessitate an examination of a more extensive collection of these fungi than has been possible here.

Literature Cited

1. CAMPBELL, R. N. 1958. Nutrient requirements for the production of perithecia by Ceratocystis variospora and other species. Am. J. Botany 45: 263-270.
2. CARMICHAEL, J. W. 1955. Lactofuchsin: a new medium for mounting fungi. Mycologia 47: 611.

3. DAVIDSON, R. W. 1935. Fungi causing stain in logs and lumber in the southern States, including five new species. J. Agr. Research 50: 789-807.
4. DAVIDSON, R. W. 1944. Two American hardwood species of Endoconidiophora described as new. Mycologia 36: 300-306.
5. DAVIDSON, R. W. 1953. Two common lumber-staining fungi in the western United States. Mycologia 45: 579-586.
6. DAVIDSON, R. W. 1955. Wood-staining fungi associated with bark beetles in Engelmann spruce in Colorado. Mycologia 47: 58-67.
7. DAVIDSON, R. W. 1958. Additional species of Ophiostomataceae from Colorado. Mycologia 50: 661-670.
8. EL-ANI, A. S. 1958. The genetics of Ceratostomella radicicola and the phylogenetic relationship between Chalaropsis and Chalara. Am. J. Botany 45: 228-232.
9. HEDGCOCK. G. G. 1906. Studies upon some chromogenic fungi which discolor wood. Missouri Botan. Garden Ann. Rept. 17: 59-114.
10. HUNT, J. 1956. Taxonomy of the genus Ceratocystis. Lloydia 19: 1-59.
11. LEACH, J. G., L. W. Orr, and CLYDE CHRISTENSEN. 1934. The interrelationships of bark beetles and blue-staining fungi in felled Norway pine timber. J. Agr. Research 49: 315-342.
12. MOREAU, C. 1952. Coexistence des formes Thielaviopsis et Graphium chez une souche de Ceratocystis major (van Beyma) nov. comb. Rev. Mycol. Suppl. Colon. 17: 17-25.
13. NELSON, R. M. 1934. Effect of blue-stain fungi on southern pines attacked by bark beetles. Phytopathol. Z. 7: 327-353.
14. RUMBOLD, CAROLINE T. 1931. Two blue-staining fungi associated with bark-beetle infestation of pines. J. Agr. Research 43: 847-873.
15. RUMBOLD, CAROLINE T. 1936. Three blue-staining fungi, including two new species, associated with bark beetles. J. Agr. Research 52: 419-437.
16. RUMBOLD, CAROLINE T. 1941. A blue-stain fungus, Ceratostomella montium n. sp., and some yeasts associated with two species of Dendroctonus. J. Agr. Research 62: 589-601.
17. SCHEFFER, T. C., and R. M. LINDGREN. 1932. Some minor stains of southern pine and hardwood lumber and logs. J. Agr. Research 45: 233-237.
18. VERALL, A. F. 1939. Relative importance and seasonal prevalence of wood-staining fungi in the southern States. Phytopathology 29: 1031-1051.
19. WRIGHT, E. 1935. Trichosporium symbioticum n. sp., a wood-staining fungus associated with Scolytus ventralis. J. Agr. Research 50: 525-538.
20. WRIGHT, E. 1938. Further investigations of brown-staining fungi associated with engraver beetles (Scolytus) in white fir. J. Agr. Research 57: 759-773.
21. ZABEL, R. A. 1953. Lumber stains and their control in northern white pine. J. Forest Products Research Soc. 3: 36-38.

DEPARTMENT OF PLANT PATHOLOGY AND BOTANY,
INSTITUTE OF AGRICULTURE, UNIVERSITY OF MINNESOTA,
ST. PAUL, MINNESOTA

PEACH "SHOT HOLE" IN ARIZONA --1959[1]

Paul D. Keener

Epiphytotics of aerial pathogens are of unusual interest in Arizona because of their rarity. The low humidity makes such occurrences all the more remarkable in the northern part of the State where "dry land farming" takes the place of irrigated crops farther south. A severe outbreak of the fungus causing shot-hole, Coryneum carpophilum (Lév.) Jauch (C. beijerinckii Oud.)[2], occurred on the peach variety Rio Oso Gem in the Oak Creek Canyon area, Yavapai County. Foliage, twigs and fruits were infected. Fruits were so marred by necrotic spots as to render them unfit for consumption (Fig. 1). Spores, although present in small amounts on fruits in nature, were much more abundant after specimens had been placed in a sterilized moist chamber for 3-4 days (Fig. 2).

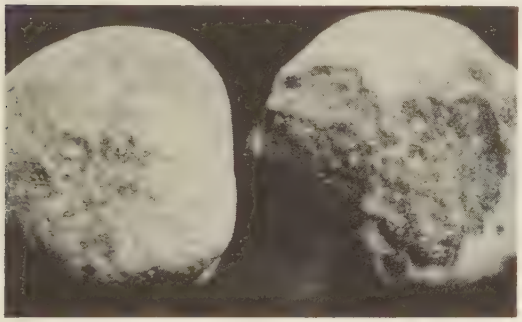

FIGURE 1. Rio Oso Gem peach fruits with necrotic lesions caused by Coryneum carpophilum (C. beijerinckii). At right lesions have coalesced to form a large scab-like area.

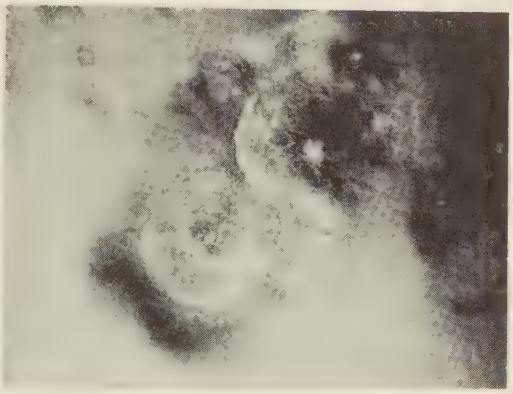

FIGURE 2. Two adjoining fruit lesions of shot-hole showing mycelium of the fungus. Fruits had been in moist chamber for 3 days.

Only one previous record for the disease on peaches (unlisted variety) exists in the files of the Department of Plant Pathology, University of Arizona, Tucson. This record was made in 1942 from observations on twig lesions from the Dos Cabezas area, Cochise County. One other record of the disease on apricot fruits was made in 1951 from the same general area as the 1959 peach report.

DEPARTMENT OF PLANT PATHOLOGY, COLLEGE OF AGRICULTURE, UNIVERSITY OF ARIZONA, TUCSON

---

[1]Arizona Agricultural Experiment Station Technical Paper No. 599.

[2]Authority names for C. carpophilum taken from Index of Plant Diseases in the United States, Part 5. p. 966, 1953.

TAPHRINA PRUNI-SUBCORDATAE (ZELLER) MIX, CAUSING WITCHES'-BROOM ON ITALIAN AND FRENCH PRUNES

Julius L. Heinis

Abstract

Prunus domestica varieties French prune and Italian prune have been found to be undescribed hosts of Taphrina pruni-subcordatae which attacks P. subcordata, the Pacific plum. On the two P. domestica varieties the organism causes witches'-broom and leaf curl

SURVEY

In the winter of 1954-55 a witches'-broom condition first came to the author's attention on the French (Agen) prune variety of Prunus domestica. The disorder was fairly widespread on French prunes growing in fence rows and in old orchards of Marion County. In the spring of 1957 the same disease was seen on Italian (Fellenberg) prunes in an old orchard near Sheridan, Oregon; more than 10 trees were found infected. In 1958 observations were made in several more prune plantings in the area surrounding Salem, Oregon and several dozen infected trees were found in old orchards. In June of 1959 a survey was made of commercial orchards of P. subcordata, Pacific plum, near Lakeview in south-central Oregon. Witches'-broom and curled leaves were abundant in one orchard.

Zeller (2) described witches'-broom on P. subcordata in 1927, but he did not record it on Italian prunes. No report of this disorder on P. domestica has been found in the literature. Specimens of the witches'-broom on Italian prunes were sent to Dr. L. F. Roth, Oregon State College, Corvallis and Dr. C. G. Shaw, Washington State University, Pullman. They diagnosed the causal organism as Taphrina pruni-subcordatae (Zeller) Mix.

SYMPTOMS

Prunus subcordata (Pacific plum): The disease produced thickened branches out of which grew numerous shoots in a thick and disorderly fashion. The brooms were 20 to 60 cm in diameter. Early in the season the leaves were thick and curled. When slices were made through these leaves, asci of Taphrina sp. were easily seen with the microscope. Leaves appearing later in the season were normal and devoid of asci. Although Mix (1) reported deformed fruits (plum pockets), none were seen in this survey.

Prunus domestica (varieties Italian prune and French prune): The branch from which a witches'-broom originated was slightly swollen and a mass of twigs grew out of the point of original infection. The witches'-brooms were often from 50 to 100 cm in diameter and their weight caused them to hang down from trees.

The early spring leaves were curled and thickened. Asci were easily recognized and classed into the genus Taphrina. A few weeks later the curled leaves dried-out and all new leaves were normal.

No fruits were borne in the center of the witches'-broom, but sometimes a healthy shoot growing out of the broom bore an occasional normal prune.

MICROSCOPICAL SYMPTOMS

The asci observed on Italian prune sat on short stalk cells and formed a thin layer over the epidermis. They were fairly regular in size and shape and averaged 26 μ in width. Ascospores, numbering eight per ascus, were oval and slightly longer than wide (about 4μ x 3μ).

Asci and spores on the two Prunus species were alike in size and shape. The morphological identity of Taphrina sp. on the two hosts therefore appeared to be confirmed. It might be noteworthy that the smallest asci of T. pruni-subcordatae described by Mix (1) were 7μ longer, the width was corresponding, but the ascospores were slightly more elliptical than those described here.

CONCLUSIONS

Although no inoculation experiment was made with Taphrina sp. on P. domestica and P. subcordata, morphological similarities leave little doubt that the Taphrina sp. on the two

Prunus species belong to the same species. Despite two variances with Mix, namely shorter asci and no fruit symptoms, the organism may be identified as T. pruni-subcordatae. Therefore P. domestica varieties Italian prune and French prune should be added to the host list of this fungus.

No experiments with control methods were made. The cutting out and burning of witches'-broom during the regular pruning operation done by orchardists is easily feasible.

Literature Cited

1. MIX, A. J. 1949. A Monograph of the Genus Taphrina. University of Kansas Science Bull. 33: 3-167.
2. ZELLER, S. M. 1927. Contributions to our knowledge of Oregon Fungi -- II. Mycological notes for 1925. Mycologia 19: 130-143.

DIVISION OF PLANT INDUSTRY, STATE DEPARTMENT OF AGRICULTURE, SALEM, OREGON

THE VIRUS CONTENT OF FLOWERING CHERRIES AT SUMMERLAND, BRITISH COLUMBIA[1]

T. B. Lott and F. W. L. Keane[2]

Abstract

The Kwanzan and Shiro-fugen varieties of flowering cherry (Prunus serrulata) being used in indexing sweet cherries for latent viruses were themselves indexed for K&S virus. Symptomless trees of both varieties were found to be infected. Symptomless Kwanzan was found to be infected also with Lambert mottle and mottle leaf, and Shiro-fugen was found to be free from Lambert mottle. There were indications that the Lambert mottle which was transmitted from Kwanzan to sweet cherry was able to spread more rapidly under experimental plot conditions than selections of Lambert mottle obtained from Okanagan Valley orchards.

INTRODUCTION

Investigation of the virus content of symptomless experimental trees of flowering cherries in British Columbia was necessitated by the discovery of the K&S virus by Reeves, Cheney, and Milbrath in 1953[3]. Prunus serrulata, varieties Kwanzan and Shiro-fugen, had been in use at Summerland for the indexing of sweet cherries for latent viruses.

MATERIALS AND METHODS

The stock of Kwanzan flowering cherry being used at Summerland was bud-inoculated into sweet cherry trees of the Lambert, Bing, and Black Republican varieties. The stock of Shiro-fugen was inoculated into Lambert only. All inoculations were made into fruiting trees in the late summer and were repeated annually until the trees were removed. Indexing was carried out in a part of the isolation orchard in which numerous selections of Lambert mottle had been present for 7 years with very little natural spread. Mottle leaf had been present to a small extent with no natural spread.

RESULTS

Kwanzan-inoculated trees of all three varieties developed K&S disease in the fruits, sometimes in the first year after inoculation. Moderately severe Lambert mottle developed in the Lambert trees. The symptoms were mostly tip and marginal burn with some ring pattern. Moderate mottle leaf appeared in a few of the Bing trees.

A Lambert tree inoculated with Shiro-fugen developed K&S disease but not Lambert mottle.

No natural spread of K&S disease or mottle leaf was observed. However, symptoms of Lambert mottle, similar to those in the Kwanzan-inoculated Lambert trees, appeared in three uninoculated Lambert trees growing close by. No other natural spread of Lambert mottle occurred during the progress of this experiment.

DISCUSSION

The indexing of Kwanzan at Summerland demonstrated that this variety can remain symptomless while carrying the viruses of Lambert mottle and mottle leaf, as well as the K&S virus. The indexing of Shiro-fugen, however, showed that the clone of this variety in use at Summerland was free from Lambert mottle.

Natural spread of Lambert mottle has been serious in some orchards in the Okanagan Valley, but no comparable natural spread of selections from these same orchards has occurred in plots of the isolation orchard, despite close planting. In 7 years preceding the indexing of Kwanzan, only two Lambert trees became naturally infected.

---

[1]Contribution No. 41, Research Station, Canada Agriculture, Summerland, British Columbia, Canada.

[2]Pathologists, Research Station, Canada Agriculture, Summerland, British Columbia, Canada.

[3]Reeves, E. L., Philip W. Cheney, and J. A. Milbrath. 1955. Normal-appearing Kwanzan and Shiro-fugen oriental flowering cherries found to carry a virus of little cherry type. Plant Disease Reptr. 39:725-726.

The three uninoculated Lambert trees, which developed symptoms of Lambert mottle while the Kwanzan indexing experiment was in progress, were all within 30 feet of the Kwanzan-inoculated Lamberts, which themselves constituted a compact group. These three trees probably became infected with virus from the Kwanzan-inoculated trees rather than from any of the other possible sources of infection. This is suggested by the position of the naturally infected trees quite close to those inoculated with Kwanzan, the similarity of symptoms, the absence of natural spread in the rest of the same plot and elsewhere in the isolation orchard in the same years, and the almost complete absence of natural spread in the preceding years. Furthermore, natural spread ceased when all Kwanzan-inoculated trees and all naturally infected trees were removed. There are therefore some indications that Lambert mottle, transmitted experimentally from Kwanzan into Lambert, can spread quite readily by natural means from Lambert tree to Lambert tree in plots where many other selections of Lambert mottle have shown little or no natural spread. However, some of these other selections had spread seriously in the commercial orchards from which they had been obtained.

Results at Summerland did not give proof of the direct natural spread to sweet cherry of any of the three viruses out of Kwanzan or of the K&S virus out of Shiro-fugen.

RESEARCH STATION, CANADA AGRICULTURE, SUMMERLAND, BRITISH COLUMBIA

VIRUS INFECTION NOT THE CAUSE OF EARLINESS IN THREE STRAINS OF ITALIAN PRUNE[1]

T. B. Lott,[2] A. J. Mann,[3] and F. W. L. Keane[2]

In 1952 three early strains of Italian prune, De Maris, Greata and Richards, were being recommended and widely planted in the Okanagan Valley in preference to the regular strain of Italian.

There was considerable uncertainty as to the exact origin of these three early strains, which had proved to be practically indistinguishable under local conditions, and no information was available to show that any one of them had actually originated as a mutation of Italian prune, although this was the generally accepted supposition. Consequently an experiment was undertaken to determine whether the earliness of these strains was due to virus infection, rather than to mutation.

In 1952 grafts of the De Maris strain were placed on one mature tree and one younger bearing tree of regular Italian prune. Two similar trees were treated with the Greata strain and two with the Richards strain. The inserted grafts were encouraged to grow.

In 1954 the experiment was expanded to include a uniform block of 40 two-year-old prunes on myrobalan roots, consisting of approximately equal numbers of the three early strains and of regular Italian. These trees, with the exception of an adequate number of checks, were reciprocally grafted in the spring of 1954. Each kind was used as scion on each of the other three, every tree containing only one combination. Either two or three trees were used for most of the combinations, but groups of six trees were used for combinations in which regular Italian was the scion.

In most instances fruit was obtained both on the scion portion and on the stock portion of the tree. Results to 1958, inclusive, showed that branches growing from the inserted grafts produced fruit typical in earliness and other characteristics. With the exception of one tree in the younger plot, there was no evidence that either an earliness or a lateness factor had been transmitted to the other part of the combination. The one exception, a regular Italian grafted on a Richards stock, showed some evidence that the Italian fruit was earlier than on the check trees. In view of the large number of trees showing negative results, it was considered that this isolated instance should be disregarded.

It is concluded from this experiment that the earliness factor in these strains is not transmissible to regular Italian prune by grafting.

RESEARCH STATION, CANADA AGRICULTURE, SUMMERLAND, BRITISH COLUMBIA

[1]Contribution No. 42, Research Station, Canada Agriculture, Summerland, British Columbia, Canada.

[2]Pathologists, Research Station, Canada Agriculture, Summerland, British Columbia, Canada.
[3]Formerly Horticulturist, Dominion Experimental Farm, Summerland, British Columbia.

LEAF ENATIONS IN APPLE INOCULATED FROM CHERRY[1]

T. B. Lott and F. W. L. Keane[2]

Rasp leaf or leaf enation of sweet cherry has been known in the dry belt of British Columbia for at least 20 years. It has been serious in widely scattered orchards, and there has been natural spread. Recently considerable variation in symptoms has been observed. Leaf enation (2) in the Okanagan Valley is believed to be the same as rasp leaf described in Colorado by Bodine and Newton (1), though no direct comparison is known to have been made. The enations are also similar to those in Pfeffingerkrankheit or Eckelrade disease of Europe.

In 1954, one seedling apple tree and two Bartlett pear trees were inoculated, by budding, with material from a Napoleon (Royal Ann) cherry tree naturally infected with rasp leaf. No enation symptoms appeared in the experimental trees up to November 1959 when they were severely cut back, leaving stubs 2 inches in diameter. Back inoculations from them into cherry, made in 1955 and 1957, have given no results.

By June 1960, the apple and pear trees had made about a foot of new growth. The pear trees remained normal. In the apple tree some shoots remained normal while others showed symptoms (Fig. 1) very similar to those of rasp leaf in cherry (Fig. 2). Affected shoots were on the stumps where the inoculated branches had been removed. The oldest leaves were full sized. They showed many interveinal thickenings and a lesser number of enations. Interveinal translucent bars, as in rasp leaf, were present to some extent. The somewhat younger leaves were smaller than normal and irregular in shape. On the lower side they carried enations, some of which had serrations and glands similar to those on the edge of a leaf. The margins of these leaves were sometimes flat and sometimes strongly waved, as in rasp leaf. Veins were sometimes considerably thickened, and as in rasp leaf the angles between midribs and veins were narrower than normal. The youngest leaves on affected shoots were sometimes narrow and strapshaped. Some narrow leaves had three nearly parallel veins. Some had marginal enations or continuous double margins.

FIGURE 1. Leaves from apple tree inoculated with rasp leaf or leaf enation virus.

[1]Contribution No. 50, Research Station, Canada Agriculture, Summerland, British Columbia, Canada.

[2]Pathologists, Research Station, Canada Agriculture, Summerland, British Columbia, Canada.

FIGURE 2. Leaves from Bing cherry tree showing rasp leaf or leaf enation.

At present there is no proof that the cherry virus has been transferred to apple. However, transmission seems probable in view of the very definite enation symptoms which are not likely to be due to any cause other than the inoculation with rasp leaf virus. Adjacent apple trees, similarly cut back but not inoculated with rasp leaf virus, produced no enations. This work is being continued.

Other experiments have given some indications that chokecherry is also a host of the rasp leaf virus, and in the wild state is naturally infected. In chokecherry some branch killing occurs, while the leaves are small, harsh, wavy along the margins, and with cleared veins but no large enations.

Literature Cited

1. BODINE, E. W., and J. H. NEWTON. 1942. The rasp-leaf of cherry. Phytopathology 32: 333-335.
2. LOTT, T. B., and H. R. McLARTY. 1942. Leaf enation. In Handbook of virus diseases of stone fruits in North America. Michigan Agr. Exp. Sta. Misc. Pub. p. 51.

RESEARCH STATION, CANADA AGRICULTURE, SUMMERLAND, BRITISH COLUMBIA

TRANSMISSIBLE CORKY PIT OF FLEMISH BEAUTY PEAR[1]

F. W. L. Keane and Maurice F. Welsh[2]

Abstract

Flemish Beauty pear fruits bear shallow skin depressions or furrows, underlaid by brown, corky, flesh tissue. Graft transmission has been effected, from diseased Flemish Beauty through Bosc to healthy Flemish Beauty. Fruits on inoculated Bosc trees have shown no symptoms.

INTRODUCTION

In 1955 the authors were asked to diagnose a disorder in fruit from a block of Flemish Beauty pear trees in a commercial orchard. Symptoms of the disorder were apparent in fruits only. The affected fruit retained its characteristic shape, but bore several to many shallow skin depressions or furrows (Fig. 1) that were underlaid by brown corky flesh tissue (Fig. 2). The browning was sometimes confined to shallow pockets immediately beneath the skin, or more frequently formed a network throughout the flesh of the fruit.

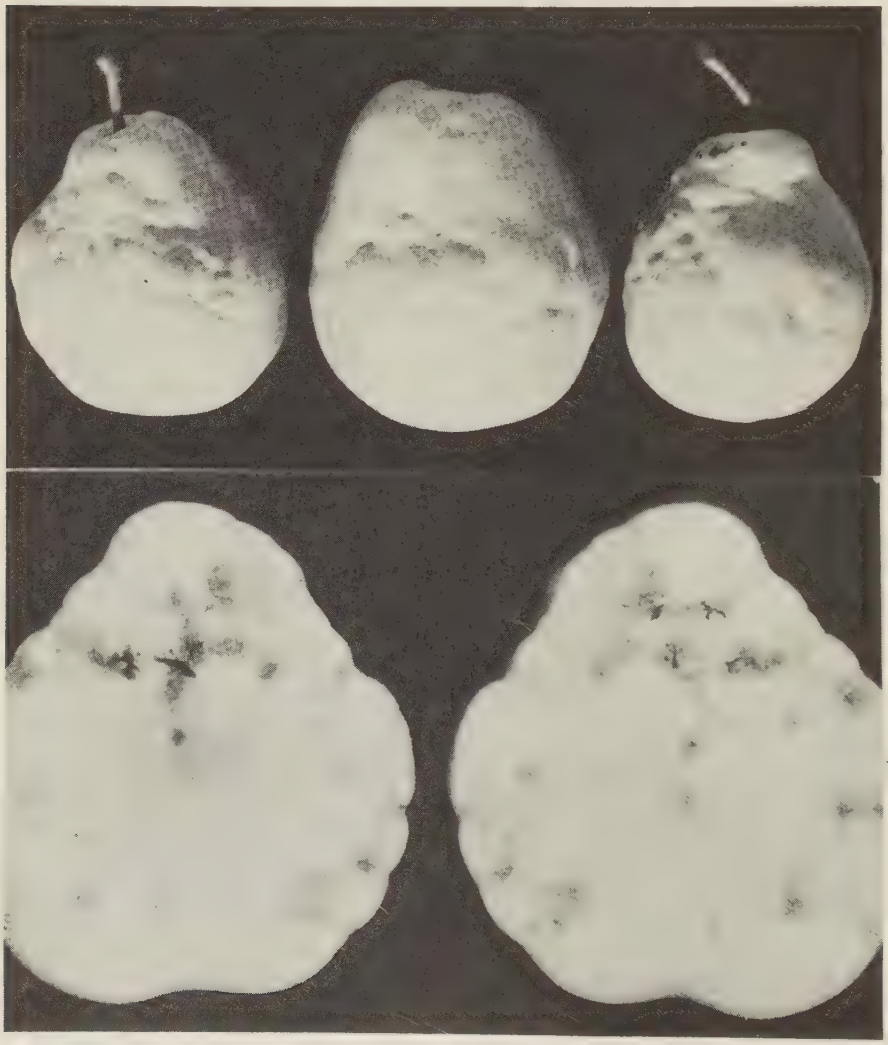

FIGURE 1. Corky pit symptoms -- pitting and furrowing of Flemish Beauty fruits.

FIGURE 2. Corky pit symptoms -- brown corky tissue in pockets beneath the skin, and in networks through the flesh of the fruit.

[1]Contribution No. 44, Research Station, Canada Agriculture, Summerland, British Columbia, Canada.

[2]Respectively Technician and Plant Pathologist.

Four of the 21 trees in the block bore severely affected fruits in 1955; 10 bore moderately affected fruits. The grower reported that severe losses from the disorder had been sustained in 1955, relatively light losses in.1952 and 1953, and moderate losses in 1954.

Several diseases have been described that cause similar symptoms in pear fruits. The symptoms bear closest resemblance to those of cork spot of Anjou pear (3) the cause of which is unknown. They also bear some similarity to the symptoms of stony pit (1, 2) which was demonstrated to be a virus disease by Kienholz, and has subsequently been reported from most pear-growing countries in the world. No report can be found of the occurrence of stony pit in Flemish Beauty, and private correspondence with Kienholz in 1955 confirmed that he had received no such report.

PROCEDURE

Four healthy bearing trees of Bosc were used as test trees for a transmission experiment. Each test tree received grafts from one of the four severely affected Flemish Beauty trees. At the same time scions of Flemish Beauty from an apparently healthy tree were applied to other limbs of the four Bosc test trees.

Scions from a Bosc tree infected with stony pit virus were applied to three healthy Bosc test trees. These trees were also top-worked with Flemish Beauty scions from the same healthy source tree.

Scions of Flemish Beauty from the healthy source tree were applied to six healthy Bosc test trees, to which no diseased pear scionwood had been applied.

RESULTS

All trees receiving grafts of infected Flemish Beauty have borne between 50 and 200 Bosc fruits per year in 1957, 1958, and 1959. Every fruit has been normal in all three seasons. Branches growing from infected and healthy Flemish Beauty grafts on all of these trees have borne fruits in one or more of the 3 years. A large percentage of the fruits on all Flemish Beauty branches have displayed typical symptoms of Flemish Beauty corky pit (Table 1). Thus the disease has been perpetuated in Flemish Beauty, and has been transmitted through Bosc to healthy Flemish Beauty; but no symptoms have been evident on Bosc fruits borne on the test trees.

Table 1. Occurrence of corky pit in Flemish Beauty fruits on test trees inoculated by spring grafting from affected Flemish Beauty trees in 1956.

Year	Tree number: 1	2	3	4
1957				
perpetuation	1/1[a]	-	-	-
transmission	-	-	-	-
1958				
perpetuation	-	7/7	-	-
transmission	-	13/13	-	-
1959				
perpetuation	1/1	19/27	4/5	2/6
transmission	5/5	55/63	3/3	3/3

[a]Affected fruits/total number of fruits borne.

Graft inoculation with stony pit from infected Bosc trees has induced stony pit fruit symptoms on all three Bosc test trees. However, the proportion of affected fruits has been very low (Table 2). The fruits displaying symptoms in 1957 were borne on spurs close to the points of graft inoculation. The affected tree from which scions were taken, selected because it showed severe symptoms in 1955, has produced only mild symptoms on a few fruits in the seasons 1956-1959.

The Flemish Beauty branches growing on the Bosc trees with stony pit did not fruit until 1959, when Flemish Beauty branches on two of the trees bore a few fruits. Five of the 17 fruits on one tree displayed typical symptoms of corky pit. None of the seven fruits on the other tree showed symptoms.

Table 2. Occurrence of stony pit in Bosc pears on test trees inoculated by spring grafting from affected Bosc trees in 1956.

Year	Tree number: 1	2	3
1957	0/47[a]	5/95	1/35
1958	5/73	8/153	0/44
1959	2/58	0/145	3/90

[a]Affected fruits/total number of fruits borne.

The six Bosc trees on which healthy Flemish Beauty was top-worked bore crops of normal Bosc fruits in all three seasons, and bore normal Flemish Beauty fruits in 1959.

DISCUSSION

The transmission tests have shown that corky pit symptoms in Flemish Beauty fruits are caused by a virus. There is some doubt whether this is the virus that causes stony pit in Bosc and other varieties, or whether it is a distinct virus.

The absence of fruit symptoms on all four Bosc trees during the three seasons that followed their graft inoculation from affected Flemish Beauty suggests that the virus in Flemish Beauty is not the stony pit virus. However, the meagre number of stony pit fruits on the Bosc trees inoculated at the same time with stony pit virus indicates that symptoms of stony pit were not severe during these seasons and makes it conceivable, although improbable, that stony pit symptoms were completely suppressed every season in the Bosc trees that had received grafts from affected Flemish Beauty.

The appearance of corky pit symptoms in Flemish Beauty fruits on one of the three trees inoculated with stony pit virus suggests several alternative possibilities. It may indicate that corky pit is caused by the stony pit virus. It may demonstrate that the affected Bosc source tree carries a second virus that causes corky pit in Flemish Beauty. There is also the possibility that this one tree has been naturally infected with corky pit virus from neighboring trees inoculated with this virus.

If corky pit of Flemish Beauty is caused by the stony pit virus, the results of these tests indicate that Flemish Beauty is more consistently affected by the virus than is Bosc, usually regarded as the most sensitive variety.

Additional tests have been initiated, in which other varieties of pear are being inoculated from Bosc stony pit sources and from Flemish Beauty corky pit sources. These tests may eventually establish whether the two diseases are caused by the same or by distinct viruses.

Literature Cited

1. KIENHOLZ, J. R. 1939. Stony pit, a transmissible disease of pears. Phytopathology 29: 260-267.
2. KIENHOLZ, J. R. 1953. Stony pit of pears. Plant Diseases; The Yearbook of Agriculture. U. S. Dept. Agr. pp. 670-673.
3. OVERHOLSER, E. L., and WALTER J. CLORE. 1937. Six years' records of amount of cork spot fruit on individual d'Anjou pear trees. Proc. Am. Soc. Hort. Sci. 34: 192-198.

RESEARCH STATION, CANADA AGRICULTURE, SUMMERLAND, BRITISH COLUMBIA

SOIL INHIBITS INFECTION WITH TOBACCO NECROSIS VIRUS[1]

C. E. Yarwood[2]

Summary

When plants were inoculated with tobacco necrosis virus by pouring a suspension of virus from infected leaves directly on the soil in which healthy plants were growing, about 10% became infected. When a suspension of infested soil was similarly used as inoculum, infection was about 3 times as great as when infected leaves were used, even though local lesion assay indicated the soil suspension contained less virus than the leaf suspension. When cucumber seeds were germinated on moist blotting paper, inoculated with a suspension of infected leaves, and maintained on blotting paper, about 77% of the plants became infected, but when these same plants were transferred to soil immediately after inoculation, only about 12% became infected. When healthy seeds or seedlings were planted in infested soil, about 83% became infected. When autoclaved or unautoclaved soil was added to inoculum prepared from leaves, the infectivity of this inoculum was reduced. To explain these results, it is believed that soil contains a virus infection-inhibiting power which is comparable to the spore germination-inhibiting power which soils exhibit toward fungi.

INTRODUCTION

Tobacco necrosis virus (TNV) is normally limited to the roots of growing plants and is normally soil-borne (8). Although natural infection of roots may occur in large numbers of plants under greenhouse (3, 7) and field conditions (4, 5, 9), artificial inoculation of roots in soil has been erratic. Price (6) reports six infections out of six inoculations when a suspension of virus was poured on soil containing tobacco roots, but Bawden (1) indicates poor transmission in soil. By contrast, inoculation of leaves of many hosts with the production of local lesions is readily accomplished (7), and inoculation of roots of seedlings in Petri dishes is moderately successful (11). The present study is an exploration of methods of inoculation of roots in soil, and confirms some of the ideas previously published.

METHODS

Cowpea leaves bearing 20 or more local lesions of TNV were ground in a mortar and diluted with about 50 parts of water. This is called leaf inoculum. Similarly, washed roots of infected plants were ground and diluted with about 50 parts of water; this is called root inoculum. For infested soil the lower part of the soil root mass of infected potted plants was shaken in about 20 parts of water; this is called soil inoculum. In the soil inoculum, roots were present and since virus is naturally released from infected roots (12) it is presumed that virus might be released from soil inoculum over an extended period.

Inoculation was usually accomplished by pouring the inoculum over the surface of soil in which test plants were growing. In some cases the roots were injured by poking a knife several times into the central areas of the pots, or by running a knife around the inside surface of the pots. In some trials the growing plants plus soil were knocked out of freshly watered pots, the root soil mass was dusted with carborundum and the inoculum was painted over the surface of the roots. To avoid soil in many trials, seeds were germinated for 2 to 5 days on moist filter paper in Petri dishes, the seedlings were shaken in a flask with a water suspension of virus and carborundum, then returned to Petri dishes or planted in soil.

The soil commonly used was a greenhouse potting mixture of steam sterilized soil, sand, and peat moss. While this soil was considered to have been once freed of most pathogens by heat, it was presumably soon contaminated with miscellaneous saprophytes and some pathogens in normal handling. In many cases natural soils were used.

Host plants consisted principally of spider plant (Cleome spinosa), bean (Phaseolus vulgaris var. Pinto, Bountiful, and Beka), cucumber (Cucumis sativus var. National Pickling),

[1]Aided by a grant (NSF-G 9120) from the National Science Foundation. The assistance of Raymond Louie and Clarence Kado in assaying most of the roots for virus is gratefully acknowledged.
[2]Professor of Plant Pathology, Department of Plant Pathology, University of California, Berkeley, California.

cowpea (Vigna sinensis var. Blackeye), tobacco (Nicotiana tabacum var. Turkish), tomato (Lycopersicum esculentum var. Bonny Best), and sugar beet (Beta vulgaris). Of these Cleome and cowpea appeared to be most susceptible but this was not clearly established. Plants such as cucumber, bean, and cowpea were usually inoculated at about 10 days after planting; other plants when they were about 3 inches high.

Since root infections by TNV normally cause no symptoms, inoculated plants were assayed by rubbing suspensions of inoculated roots on cowpea leaves. One or two cowpea leaves were used for the assay of the roots of each plant. If two or more lesions resulted, it was concluded that the root was infected, whereas if one or no lesions formed, it was concluded for statistical purposes of this study that the root was not infected. That lesions produced from these assays came from root infections and not from the original inoculum is indicated by the following: 1) the half life of the virus under conditions of these tests was about 3 days (12) whereas assays were usually not made until about 16 days after inoculation; 2) routine assays of different roots receiving the same inoculum was usually 30 or more lesions or none; and 3) reassays of positive or negative infections usually gave similar numbers of assay lesions.

The TNV used was principally a strain isolated from soil from Salinas, California.

In tests of soil moisture on infection, plants subjected to regular watering were inoculated by pouring the inoculum on the soil. This type of inoculation resulted in complete saturation of the soil. From this time on the plants to be at medium soil moisture were subjected to routine watering, those to be at low soil moisture were watered lightly only as the plants approached incipient wilting, and those to be at high soil moisture were given extra watering and saucers were placed under the pots to prevent complete escape of the drainage water. This watering regime was maintained for only 4 to 7 days, after which all plants were subjected to regular watering. At the time of assay, 10 to 20 days after inoculation, the plants at low soil moisture were only slightly smaller than those at high or medium soil moisture.

RESULTS

Of 5808 plants which were experimentally inoculated, and of which the roots were individually assayed, 1292 or 22% became infected. These plants were distributed into 360 trials which usually included 12 to 20 plants each. Some of the categories of inoculation pertinent to

Table 1. Methods of inoculation of roots with tobacco necrosis virus.

Method of inoculation	Number of plants assayed	% of plants infected
Natural (uninoculated controls)	595	1.8
Leaf inoculum added to soil	1104	10
Soil inoculum added to soil	1282	28
Shake inoculation of all seedlings, assayed from dishes	668	52
Shake inoculation of all cucumbers, assayed from dishes	143	70
Grouped comparisons of inoculum (7 trials)		
Leaf inoculum added to soil	49	4
Root inoculum added to soil	28	18
Soil inoculum added to soil	59	63
Grouped comparisons of substrate after inoculation (7 trials)		
Paper substrate in Petri dishes	30	77
Soil in pots	34	3
Grouped comparisons of seedlings inoculated by shaking and transferred to soil (8 trials)		
Transferred to soil immediately after inoculation	41	20
Transferred to soil 1 day after inoculation	29	62
Transferred to soil 2 days after inoculation	38	100
Grouped comparison of soil moisture effect (5 trials)		
Low soil moisture	20	0
Medium soil moisture	15	46
High soil moisture	21	67

this report are given in Table 1. The first five lines of data are totals of many trials over about 1 year in which the five methods of inoculation were not necessarily compared in the same trial. In this part of the table trials in which no infection resulted are included. The data given under grouped comparisons contain only data in which the methods of treatment were compared in the same test and in which some method of inoculation gave infection. Inoculations of roots in soil have sometimes failed by every method tested. These data indicate that infected roots were about 3 to 6 times as effective as a source of inoculum as were infected leaves and that infested soil was 3 to 16 times as effective as infected leaves. This was apparently not because the inoculum from soil contained more virus. In many trials inoculum from leaves applied to leaves gave more lesions than comparable inoculum prepared from soil. In one trial the numbers of lesions were 118 from leaf inoculum and 4 from soil inoculum. Wounding of roots with a knife before or after inoculation or painting the virus on the roots after dusting them with carborundum was not clearly superior to pouring the inoculum on the soil of pots with uninjured roots.

Inoculation of seedlings without soil by the shaking method was about 5 times as effective as inoculation of roots in soil with the same inoculum, and with cucumber, about 7 times as effective. In seven tests the infection of cucumber seedlings which were inoculated by shaking and maintained on moist paper in Petri dishes was compared with that of seedlings which were inoculated in the same way but transplanted to soil immediately after inoculation. Those maintained on paper yielded 28 times as many infected seedlings as those transferred to soil.

In 11 trials independent of the above, cucumber seedlings which had germinated for 3 days on paper were inoculated by shaking, transferred to soil at different times after inoculation, and assayed at about 20 days after inoculation. Controls for each trial consisted of four seedlings inoculated as above but assayed directly from Petri dishes at about 6 days after inoculation. Only eight trials yielded 100% infection of the controls and only data from these eight trials are included in Table 1. The infection of plants held on paper for 1 day after inoculation was 3 times as great as that of seedlings transferred to soil immediately after inoculation, and infection of plants held on paper for 2 or more days after inoculation was 5 times that of seedlings transferred to soil immediately after inoculation.

To measure the effect of soil on infection of leaves, the infection resulting from leaf inoculum was compared with the infection from this same inoculum to which soil was added. Twenty % by weight of air dried field soil, and of the same soil after autoclaving, was added to different lots of virus suspension, allowed to stand 24 hours, filtered through cheesecloth, and used as inoculum. The number of lesions from the control inoculations (two replications in each of three trials) was 672, the number of lesions resulting from the addition of unsterilized soil was 31% of the controls, and from the addition of sterilized soil was 48% of the controls.

DISCUSSION

Several independent approaches indicate that some soils inhibit infection of roots by tobacco necrosis virus. This is most clearly indicated where inoculated seedling roots show heavy infection when maintained in Petri dishes apart from soil, but show very poor infection when transplanted to soil immediately after inoculation. The poorer infection when infected leaves are used as a source of inoculum than when infested soil is used is an indirect type of evidence which may indicate that the infection inhibiting power of the infested soil is saturated with virus.

This virus infection-inhibiting power of soil resembles fungistasis of soil (2) in that inhibition of virus infection and inhibition of spore germination are directed against pathogens which normally inhabit the soil. Fungistasis could logically confer survival value on the fungus spores in the absence of susceptible roots, and a similar phenomenon could apply to viruses, though the writer knows of no information to support it.

If the present hypothesis is correct, success in inoculation of roots in soil will increase as the ratio of virus to inhibitor (soil) is increased, and perhaps to lesser extent as the ratio of roots to soil and to virus is increased. Support for this derives from three sources. As the concentration of virus is increased, the amount of infection is increased (6). This could logically be a direct dilution effect, but it could be that increasing concentration of virus saturates the infection-inhibiting powers of the soil. That infection increases as the ratio of root to soil increases is supported by the high TNV infection observed by Frazier (3) when strawberry roots extending through the drainage holes of pots of soil contacted the contaminated bench tops.

Two factors which were not controlled in most of these tests but which are believed to be important in infection with TNV are temperature and soil moisture. TNV is favored by temperatures of about 22°C (10) and temperatures much above this prevailed during much of this study.

High soil moisture was shown in this study to favor infection, but was uncontrolled in most trials and is believed to have been frequently too low for infection.

According to this study, high infection with TNV can be secured by planting seeds or plants in infested soil, or by incubating the inoculated seedlings apart from soil for 2 days before planting in soil. These studies do not indicate how natural infection occurs, and no satisfactory method of inoculating healthy plants in virus-free soil has been devised. The question of whether natural inoculation is effected by mechanical means or by means of a living vector is unresolved.

Literature Cited

1. BAWDEN, F. C. 1953. Some properties of the tobacco necrosis viruses. Proc. 7th Int. Botan. Congr. Stockholm. 1950: 705-706.
2. DOBBS, C. G., and W. N. HINSON. 1953. A widespread fungistasis in soils. Nature 172: 197-199.
3. FRAZIER, N. W. 1955. Tobacco necrosis virus on strawberry. Plant Disease Reptr. 39: 143-147.
4. FRY, P. R. 1952. Note on the occurrence of a tobacco necrosis virus on the roots of lettuce showing big-vein. New Zealand J. Sci. Tech. Series A. 34: 224-225.
5. HOLLINGS, M. 1960. Unpublished observations.
6. PRICE, W. C. 1938. Studies on the virus of tobacco necrosis. Am. J. Botany 25: 603-612.
7. SMITH, K. M. 1937. Studies on a virus found in the roots of certain normal-looking plants. Parasitology 29: 70-95.
8. SMITH, K. M. 1957. Textbook of plant viruses. Little, Brown., Boston. 652 pp.
9. TOMLINSON, J. A., and B. R. SMITH. 1958. Big vein disease of lettuce in Britain. Plant Pathology 7: 19-22.
10. YARWOOD, C. E. 1952. Latent period and generation time for two plant viruses. Am. J. Botany 39: 613-618.
11. YARWOOD, C. E. 1959. Virus increase in seedling roots. Phytopathology 49: 220-223.
12. YARWOOD, C. E. 1960. Release and preservation of virus by roots. Phytopathology 50: 111-114.

DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA

CROWN ROTS AND BOTRYTIS FLOWER BLIGHT OF STATICE[1]

Curtis R. Jackson

Two species of statice, Limonium sinuatum and L. bonduellii (Plumbaginaceae), are commonly cultivated on a commercial scale in Florida. Approximately 50 acres of a mixture of the two species are grown annually. Several serious diseases, apparently unreported on these hosts, cause considerable loss to growers throughout the 9-month period (August-April) during which the crop is grown. This report presents symptoms and results of pathogenicity studies of the diseases.

CROWN ROTS

A rosette growth habit is typical for both host species during all stages of growth and true stems are commonly less than 5 inches in length. The numerous leaves radiating from the stem are appressed to the soil, forming a dense ground cover that hinders moisture loss from the soil. Most large statice plantings are irrigated by seepage from surrounding irrigation ditches or buried tiles. Crown rots are serious disease problems and consistently result in an estimated 5 to 10% loss of plants during the year. Occasionally losses of 30% have been observed.

Crown rots are caused by the pathogens Sclerotium rolfsii Sacc. and Rhizoctonia solani Kuehn. Isolations from diseased plants generally yield only one of these fungi but both are occasionally isolated from a single plant. The results of a 2-day survey in four separate growing areas indicated that both diseases were equally frequent, even within the same field. Field symptoms of the diseases are similar and the diseases cannot be reliably distinguished unless signs of the pathogen are present. The initial symptom of infection is a slight wilting which becomes progressively more severe until the plant collapses. Concomitantly, the lowermost leaves become reddish-brown and die. At this stage in the symptom sequence, the crown of the plant decays and the top can be easily removed from the roots (Fig. 1).

Sclerotium Crown Rot: Initially, the pathogen attacks the leaf petioles and midribs that lie on or near the soil, causing watery, reddish lesions. The infection progresses to the petiole base and thus to the stem of the plant. Direct infection of crown tissue from the surrounding soil also occurs; unilateral infection has rarely been noted. Sclerotia are often produced abundantly on necrotic surfaces.

The pathogenicity of three isolates was demonstrated by using sclerotia from pure cultures. The sclerotia were placed on the soil surface 3 inches from mature potted plants that were misted intermittently. Within 14 days mycelium of the pathogen had grown over the soil surface, infected the host, and produced sclerotia on the necrotic petioles. Control plants in uninfested soil remained healthy.

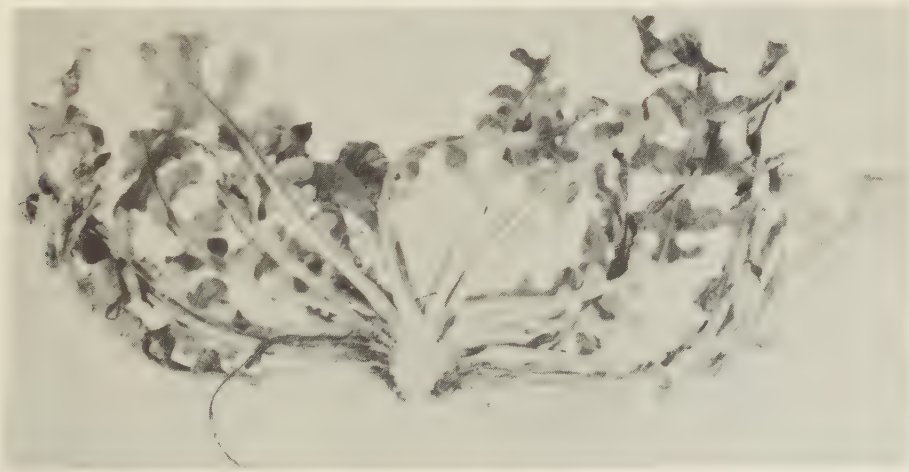

FIGURE 1. Sclerotium crown rot of statice showing top of plant which has separated from roots at the soil level.

[1]Florida Agricultural Experiment Station Journal Series, No. 1100.

Rhizoctonia Crown Rot: Water-soaked, gray lesions are first seen on leaf petioles and midribs lying on or near the soil. The lesions elongate along the petioles to the stem. Direct infection of the crown from the surrounding soil occurs less frequently than in the case of S. rolfsii and unilateral infections are more common. At times, lysigenous cavities develop in the stems of old plants due to the activities of the pathogen. Lower leaf blade infection, which is frequently seen, produces irregular, orange, sunken lesions, 2 to 12 mm in diameter.

The pathogenicity of two isolates of R. solani was demonstrated in the same manner as given for S. rolfsii except that 1-cm discs of a PDA culture were used to infest the soil.

In greenhouse pathogenicity tests, under conditions favorable for growth of both pathogens, plants of the two host species were equally susceptible to S. rolfsii and R. solani. The pink, white, rose, and lavender varieties of L. sinuatum were also equally susceptible. These re-results differ from observations of growers, some of whom aver that the rose or lavender L. sinuatum, and L. bonduellii, are unmistakably more susceptible in the field.

BOTRYTIS BLIGHT

A third disease of statice, caused by Botrytis cinerea Fr., has been known to growers for many years. The pathogen attacks the inflorescences of plants and rapidly renders them commercially valueless. The largest individual planting observed during 1960, in which all flowers were infected, was 2.5 acres.

Symptoms: Infection of inflorescences commonly begins with the collapse and discoloration of the showy membranous calyces and associated interior bracts. The pathogen grows rapidly through the compact flower clusters and as flowers dry out they drop from the inflorescence rachis (Fig. 2). The uppermost extensions of the scape wings become infected and brown. The infection progresses rapidly downward, eventually killing most of the branches of the panicle. Sporulation is abundant on all necrotic surfaces under favorable moisture and temperature conditions.

FIGURE 2. Panicle branches of L. bonduellii showing infected flowers and bare branch tips on the right and uninfected flowers on the left.

Lower, large inflorescence branches may develop discrete lesions which eventually girdle the branches, leading to the death of all distal portions of the panicle. Such branch lesions are slightly depressed, tan to orange, up to 2 cm in length, and show faint dark banding. Sporula-

tion is abundant on the necrotic surfaces.

Growth in Culture: The optimum temperature for growth in vitro was determined by culturing the fungus in 500-ml Erlenmeyer flasks for 9 days at constant temperatures from 12° to 36° C in 4° C increments. One-hundred ml of a liquid medium containing V-8 juice, ammonium nitrate and dibasic potassium phosphate were used in each flask. The contents of each flask were collected on a previously weighed filter paper. The dry weight of mycelia was maximum at 20° C and mycelial weight at 16° and 24° C decreased in this order. Weights of mycelia at 20°, 16° and 24° were all significantly different at the 5% level from each other and from all other temperatures.

Pathogenicity: Isolates of B. cinerea from infected flowers were obtained in pure culture and the conidia were used for inoculation tests. Large bunches of flowers of both species of all colors of L. sinuatum were inoculated by atomizing with an aqueous suspension of conidia. Flower bunches were immediately placed in large polyethylene bags which were then sealed. One such bag and a bagged control bunch which had been sprayed with sterile water were placed in each of five constant-temperature chambers. The chambers were maintained at 12°, 16°, 20°, 24° and 28° C for 3 days, after which disease ratings were made.

Slight flower infection occurred at 12° and 16° C. Moderate flower infection was noted at 20° and 28° and heavy infection of flowers, bracts, and scape wings occurred at 24°. Calyces of flowers in the control bunch incubated at 28° had become flaccid after 3 days. This condition was not found to be due to the pathogen but was apparently caused by incubation under conditions of high humidity and temperature. All other control bunches appeared normal. Slight resistance to infection was displayed by a lavender (Lavender Queen) and a rose variety of L. sinuatum. Flowers of L. bonduellii were very susceptible to the pathogen. These susceptibility ratings are in close agreement with field observations.

GULF COAST EXPERIMENT STATION, UNIVERSITY OF FLORIDA,
BRADENTON

THE SUSCEPTIBILITY OF TWENTY-THREE TREE SPECIES TO BLACK ROOT ROT

Samuel J. Rowan[1]

Abstract

Seedlings of 22 coniferous and one hardwood tree species were grown in infested soil under optimum greenhouse conditions for severe black root rot development. As a result of the test, 11 Pinus species were rated susceptible. Pinus glabra was the only pine classified as highly resistant. Three other coniferous species and Liquidambar styraciflua were rated as immune, two conifers were highly resistant, and the seedlings of six coniferous species died from severe root rot and/or high soil temperature before completion of the test.

Reports of root rots affecting seedlings in a number of southern forest nurseries have been conflicting in regard to the relative susceptibility of species. In a report of the root rot that occurred at the Ashe Nursery in Brooklyn, Mississippi, Henry (1) stated that slash pine was relatively tolerant to the disease, but loblolly, longleaf, and shortleaf pines were seriously affected. In a survey of southern nurseries Hodges (2) reported slash and loblolly pines as the species most seriously affected by black root rot. His observations over a wider area partially reflect the relative abundance of these two species in southern nurseries and leave the relative susceptibility of different species under identical conditions in doubt. The black root rot referred to herein is described in detail by Henry (1) and Hodges (2).

This study was made to determine which species were more seriously affected and which, if any, could be grown successfully in soils known to have a high black root rot potential.

EXPERIMENTAL PROCEDURE

In February 1959, 115 six-inch clay pots, enough for five replications for each species, were filled with soil from areas in the Horseshoe Bend Nursery, Glenwood, Georgia, known to be heavily infested with Sclerotium bataticola Taub. and Fusarium spp., the organisms causing black root rot. The following species were established in pots in a greenhouse: Pinus elliottii, P. palustris, P. taeda, P. echinata, P. strobus, P. resinosa, P. serotina, P. clausa, P. edulis, P. glabra, P. taeda x echinata (F-2 hybrid), P. halepensis, P. laricio, P. maritima, P. virginiana, Juniperus virginiana, Chamaecyparis thyoides , Cupressus arizonica, Taxodium distichum, Pseudotsuga menziesii, Abies fraseri, Picea engelmannii, and Liquidambar styraciflua. All pots were buried to within 1 inch of their tops in the bench soil.

After 1 month, seedlings were thinned to five per pot and the temperature of the bench soil increased to 85° to 90°F with heating cables. This temperature range was maintained throughout the experiment to provide optimum conditions for infection by the root rot fungi (2).

After 9 months the numbers surviving and root rot index (an arbitrary scale from 0 to 9 representing the percentage of the root system occupied by lesions, with a rating of 9 equaling 100% infection) were recorded. Four root pieces were selected from four plants of each species, surface sterilized for 1 minute with a 5.25% solution of sodium hypochlorite, and plated on potato-dextrose agar. The number of root pieces yielding Fusarium spp. and/or Sclerotium bataticola was recorded after incubation at room temperature for 4 days.

RESULTS AND DISCUSSION

The root systems of the four species rated as immune (Table 1) were well developed and failed to show any signs of damage due to black root rot. The fact that Fusarium spp. were isolated from two species in the immune group might be explained by incomplete surface sterilization.

Pinus glabra, which was rated as highly resistant, was the only one of 12 pine species which did not suffer serious root damage. The other 11 species of pines, which include all of the species grown on a large scale in southern nurseries, were rated susceptible to black root rot. Of the four most important southern species, slash pine was rated least susceptible, with a root rot index of 5.28. Longleaf pine was rated second, with an index of 8.20; and loblolly and shortleaf pines were rated most susceptible, with a 9.00 index.

[1]Plant Pathologist, U. S. Forest Service, Macon, Georgia in cooperation with the Georgia Forest Research Council.

Table 1. Relative susceptibility of certain tree species to black root rot.

Species	Root rot index (rating)	% seedling survival	Seedling roots yielding: Fusarium spp. (%)	Seedling roots yielding: S. bataticola (%)
Immune species:				
Cupressus arizonica	0.00	68	25	0
Taxodium distichum	0.00	100	25	0
Chamaecyparis thyoides	0.00	100	0	0
Liquidambar styraciflua	0.00	100	0	0
Highly resistant species:				
Juniperus virginiana	1.27	100	25	0
Pinus glabra	2.64	100	25	0
Susceptible species:				
Pinus elliottii	5.28	96	100	0
Pinus taeda x echinata (F-2 hybrid)	7.00	100	100	0
Pinus palustris	8.20	76	100	0
Pinus maritima	8.85	88	75	0
Pinus virginiana	9.00	100	100	0
Pinus serotina	9.00	44	100	0
Pinus clausa	9.00	44	100	25
Pinus halepensis	9.00	64	100	75
Pinus taeda	9.00	88	100	50
Pinus echinata	9.00	28	100	0
Pinus laricio	9.00	20	100	50
Species which failed to survive:				
Abies fraseri	9.00	0	75	25
Pseudotsuga menziesii	9.00	0	75	25
Picea engelmannii	9.00	0	100	25
Pinus strobus	9.00	0	100	0
Pinus resinosa	9.00	0	100	0
Pinus edulis	9.00	0	100	0

Even though the differing species have been listed in order of their respective susceptibilities to black root rot in this report, their susceptibility may change with changing environmental conditions. A lower soil temperature, for example, may enable certain species to resist the pathogens more effectively than other species. While the cause of death of those species which died before completion of the study was not determined, the root symptoms indicated severe root infection.

The presence of Fusarium spp. on the species listed as immune, or the absence of S. bataticola on the susceptible species, as indicated by isolations does not necessarily indicate the relative pathogenicity or abundance of the root rot fungi. Since S. bataticola damages the roots from the outside without entering the root proper (2), surface sterilization is very likely to eliminate it from root pieces. The presence of a Fusarium on root pieces may be an indication of the lack of complete surface sterilization, recontamination, or the susceptibility of the plant to fungal attack. However, isolation of the root rot fungi from root pieces does show that the fungi were present and root rot symptoms indicate their presence in sufficient quantities to cause black root rot.

In nursery areas where black root rot is prevalent, only spruce pine can be grown with any assurance of obtaining a successful pine crop, without soil fumigation. Redcedar, or any of the species listed as "immune," may also be grown in black root rot infested soil with success, but all of the tree species listed as "susceptible," including slash pine, must be considered too susceptible to be grown in soil with a high black root rot potential unless the beds are fumigated prior to sowing.

Literature Cited

1. HENRY, B. W. 1953. A root rot of southern pine nursery seedlings and its control by soil fumigation. Phytopathology 43: 81-88.
2. HODGES, C. S. 1959. Studies on black root rot of pine seedlings. Ph.D. Thesis, University of Georgia. 98 pp.

FOREST SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, MACON, GEORGIA

A METHOD OF EVALUATING FUNGICIDES IN THE SOIL UNDER CONTROLLED CONDITIONS

J. H. Reinhart[1]

Abstract

A test of potential soil fungicides using Fusarium oxysporum f. cucumerinum and cucumber plants as the host-disease complex has been developed. Rigid control of environmental factors and a new method of data calculation result in high reproducibility and simplicity of technique. The method is particularly adapted to comparison of closely related compounds of similar fungitoxic activities.

Methods to evaluate chemicals for use as soil fungicides have received much attention in recent years and several techniques have been developed. Arndt (1) tested chemicals for control of Rhizoctonia incited damping-off of cotton in artificially infested sand. Kendrick and Middleton (9) and Zentmyer (15) have developed testing techniques for evaluating chemicals on a laboratory scale which have become standard methods in soil fungicide studies. Methods of testing chemicals in-the-furrow on a greenhouse scale have been described by Sinclair (12, 13) and Ranney and Bird (11). A discussion of the use of several test methods and their interpretation relating to fungicide performance has recently been presented by Domsch (7).

The greenhouse test methods employing pathogen and host plant in soil have been developed primarily for evaluation of a small number of compounds which are nearly ready for field testing. For the purpose of screening a large number of candidate chemicals which, in some cases, do not differ greatly in activity, a method has been developed which is simple to conduct and is highly reproducible. The advantages of the method from a screening standpoint are that it produces the same high incidence of disease in each test, and yields reproducible disease control by a given chemical. The numerical values used to express chemical effectiveness permit valid comparisons between tests using a single expression which bears a constant relation of chemical effectiveness.

The method to be described involves the use of the Fusarium disease of cucumber, which has proven most satisfactory in our laboratory. The technique is a modified version of one developed by investigators at the Ohio State University Research Foundation.

METHODS

The pathogen used is Fusarium oxysporum f. cucumerinum with Early Green Prolific cucumber as the host plant. The organism is grown on sterilized oats in 1/2-pint milk bottles. Seventy-five grams of oats plus 53 milliliters of water are autoclaved in each bottle and infested with 1-2 grains of oats from a previous culture. These cultures are ready for use after 10 to 14 days' incubation at 25°C.

The chemical to be tested is formulated as a 10% dust using a mixture of pyrophyllite (Pyrax ABB) and synthetic calcium silicate (Micro-Cel) (8:1) as a diluent. Formulation is accomplished by simple blending of ingredients in 8-ounce ointment jars with six to eight 3/4-inch ceramic balls on a jar-roller; or, in the case of tacky or liquid materials, by impregnating a solvent containing the chemical on the diluent, evaporating the solvent, and blending in jars as above.

In any single test a chemical may be tested at one or several concentrations. For any one concentration 3000 g of air-dried, steam sterilized soil plus 60 ml of water are placed in an 8-quart Patterson-Kelley intensifier model shell blender. It has been found that the water prevents the annoyance of dust in subsequent operations and results in soil of suitable consistency for easy handling. To this 3000 g of soil are also added 18 g of the 10- to 14-day-old oat culture of the pathogen. Finally, the 10% dust formulation of chemical is added in the amount required to give the desired concentration based on the dry weight of the soil. This mixture is blended for 3 minutes. In each test, similar portions of soil lacking chemical and with and without the Fusarium oat culture are blended to serve as infested and uninfested controls.

[1]Plant Physiologist, Olin Mathieson Chemical Corporation, Port Jefferson Station, New York.

From 3000 g of dry soil thus treated, 660 g are placed in each of three pint paper containers[2]. The soil is tamped flat, and 15 cucumber seeds are put into each container. The seeds are covered with 140 g of soil from the remainder of the original 3000 g. Three containers are prepared for each treatment. The soil is compacted over the seeds and the containers are placed in a constant environment room under fluorescent lights at a temperature of 80°F, the optimum for development of Fusarium wilt symptoms (2, 4, 10). One hundred ml of water are added to each container to bring the moisture content to 80% of field capacity for the particular soil which we use, a mixture of sand and sandy loam. Each container is weighed daily, and when its weight is less than 838 g, or 30% of field capacity, water is added to bring it back to the 50% of field capacity. Thus, the soil moisture content of the containers is kept in the range of the optimum for development of the organism (14).

In this test, no pre-emergence damping-off occurs. Fusarium damage is evidenced entirely as post-emergence seedling wilt. Each test is terminated when the stand in the infested controls has diminished to 10 to 15%. This is routinely 15 to 20 days after the test has been set up.

At this time the stand of living, and apparently healthy, plants in each container is recorded. The total stand for each treatment is calculated. Using each of the three containers in each treatment as a replicate, an analysis of variance can be made. However, this is not ordinarily done, as the replicates usually agree very closely.

The percentage stand based on the total seeds planted is calculated. Then the percentage kill attributable to the fungus in each treatment (including infested control) is calculated according to the following formula:

$$\frac{A - B}{A} \times 100$$

Where A = % stand in uninfested controls
B = % stand in treatment

The percentage control by any chemical treatment is then calculated by the formula[3]:

$$\frac{C - D}{C} \times 100$$

Where C = % kill in infested control
D = % kill in chemical treatment

DISCUSSION

The efficiency and reproducibility of the method depend primarily upon the manipulations required to keep the environmental factors of moisture and temperature equal in all containers in a test. The weighing of the soil at each stage of setting-up of the test is facilitated if a direct reading balance is available, although an Ohaus triple beam balance has proven satisfactory. The additional effort required to perform these weighings, and those attendant on the daily waterings, is compensated by the high reproducibility of results, the very infrequent occurrence of test failures, and the reduction in number of replicates required.

It is probably true that the addition of an amount of water to the pint containers to bring the entire soil volume to 50% of field capacity does not actually accomplish this because the upper level of soil is close to saturation while the lower portion is below the desired moisture content. However, in practice, the technique has given satisfactory results, and the important factor is uniformity of moisture levels among containers.

In testing new chemicals, it is advisable to include an uninfested, but chemically treated series. The results of such treatments are not included in calculation of disease control, but serve as measure of phytotoxicity. If stand reductions occur in these treatments, it is known that apparent lack of control in infested treatments may be due to chemical toxicity and lower chemical concentrations should be tested to determine if adequate disease control is obtained

---

[2]Sealtest Nestrite containers No. 1716-S may be used. Waxed containers are preferable because they minimize water loss, but unwaxed ones can be used satisfactorily.

[3]The writer wishes to acknowledge the assistance of Mr. David Shermer in developing the method of calculation of disease control.

at non-phytotoxic rates.

A particularly important feature of the method is the manner of calculating the disease control, which can be applied to tests run by any technique as long as chemically treated infested soil, untreated infested soil, and untreated sterile soil are included in the test.

The problems encountered in the interpretation of data from testing of the action of fumigants against pathogens in the soil have been discussed by Bald and Jefferson (3). While their discussion deals with tests conducted under field conditions, the factors responsible for variability in their work are important in controlled studies in the laboratory. They point out that variability in results may derive from the presence of more than one pathogen with resulting interactions, the variation in inoculum or level of infestation in the soil and the heterogeneous mixing of chemical throughout the soil medium. In addition, chemicals which may appear quite effective under conditions of moderate infestation may fail to give adequate control where inoculum potential is greatly increased. Each of these factors has been considered in the development of this method and the techniques are designed to control each variable. The use of steam-sterilized soil assures the presence of only one pathogen; the careful measurement of added inoculum and control of moisture level and temperature result in a standard and high infestation level; and mixing of chemical with soil mechanically in a standard manner leads to homogeneous distribution in the soil. It has been found that control of these factors is extremely important for reproducibility.

The importance of the method of calculation of results lies in the fact that the percentage control value is an expression of the effect of the chemical in preventing development of disease among the portion of the plant population which would become diseased due to the added pathogen. In a population of seeds planted under circumstances in which disease will develop, there are three possible classes of individuals: 1) those which would not survive due to causes other than the pathogen -- the demise of this group is sometimes termed "natural mortality;" 2) the group which dies due to the direct cause of the pathogen; and 3) the group which survives despite the presence of the pathogen. Since the percentages of plants in groups 1 and 3 usually vary from test to test, any value based upon total survival of normal plants will not give a true comparison of chemical effect between tests. The important value is a measure of the number of plants which survive which would have been diseased if the chemical had not been present. By determining percentage kill by Formula 1 the "natural mortality" factor is eliminated. Then calculating percentage control as related to the percentage kill in the infested checks by Formula 2, a true value for chemical performance is obtained which is not affected by the number of plants which would have survived even though the chemical was not present.

Table 1. Reproducibility of test data in four tests of the manganese salt of pyridinethione[a] for control of Fusarium wilt of cucumber.

Test number	Chemical	Concentration (ppm)	Total stand	% stand	% kill	% control
1	Mn salt of pyridinethione	50	39	87	11	87
	do.	25	33	73	26	70
	Infested check	--	6	13	86	--
	Uninfested check	--	44	98	--	--
2	Mn salt of pyridinethione	50	39	87	11	87
	do.	25	33	73	26	70
	do.	12.5	33	73	26	70
	Infested check	--	6	13	86	--
	Uninfested check	--	44	98	--	--
3	Mn salt of pyridinethione	50	30	67	26	70
	do.	25	28	62	32	64
	Infested check	--	5	11	88	--
	Uninfested check	--	41	91	--	--
4	Mn salt of pyridinethione	25	31	69	21	76
	do.	12.5	23	51	41	53
	Infested check	--	5	11	87	--
	Uninfested check	--	39	87	--	--

[a]Manganese Omadine.

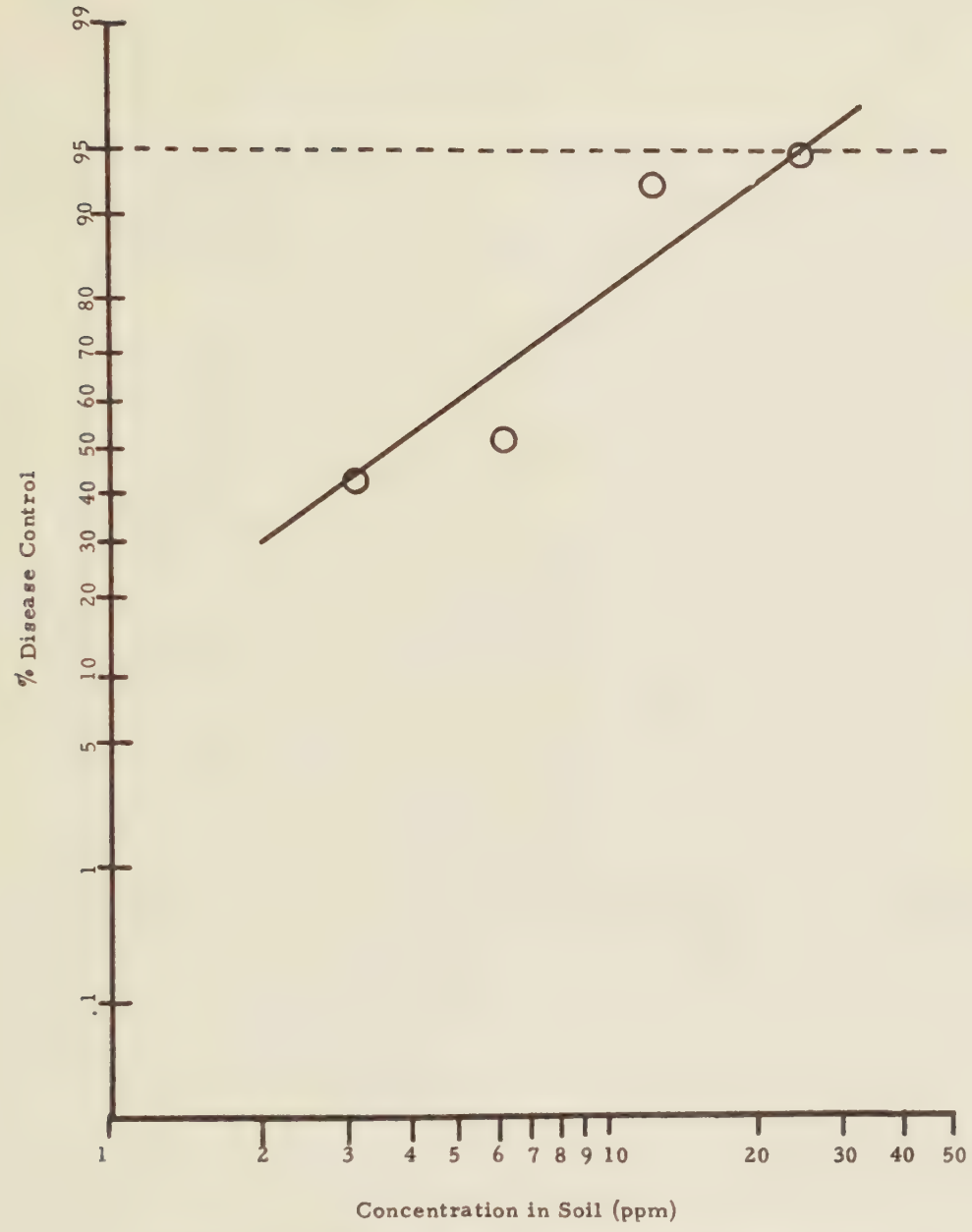

FIGURE 1. Dosage-response - Control of Fusarium disease of cucumber by zinc salt of pyridinethione.

The results of four tests for Fusarium control by the manganese salt of 2-pyridinethiol-1-oxide (Manganese Omadine (5)) are presented in Table 1. The data illustrate that relative disease control values which are highly reproducible can be obtained against Fusarium under laboratory conditions. This requires careful control of environmental factors and calculation of results in a way which eliminates individuals in "natural mortality" and "survivor" groups.

Dosage-response curves are commonly used for comparing activities of chemicals as foliar fungicides. The theory and application of dosage-response curves plotted on logarithmic probability paper are discussed by Bliss (6) and their application to studies of fungicides has been reviewed by Horsfall (8). While no detailed statistical study of the application of dosage-response curves to data obtained by this method has been carried out, plotting of the data on log-probit paper has been useful in comparing the fungitoxicity of several compounds. A typical dosage-response for the zinc salt of pyridinethione (5) is illustrated in Figure 1.

Literature Cited

1. ARNDT, C. H. 1953. Evaluation of fungicides as protectants of cotton seedlings from infection by Rhizoctonia solani. Plant Disease Reptr. 37: 397-400.
2. ARNDT, C. H. 1957. Temperature as a factor in the infection of cotton seedlings by ten pathogens. Plant Disease Reptr. Suppl. 246, Aug. 15.
3. BALD, J. G., and R. N. JEFFERSON. 1956. Interpretation of results from a soil fumigation trial. Plant Disease Reptr. 40: 840-846.
4. BEACH, W. S. 1949. The effects of excess solutes, temperatures and moisture upon damping-off. Pennsylvania State College, Agr. Exp. Sta. Bull. 509, 1-29.
5. BERNSTEIN, J., and K. A. LOSEE. 1958. Heavy metal derivatives of 1-hydroxy-2-pyridinethiones and method of preparing same. U. S. No. 2,809,971.
6. BLISS, C. I. 1941. Biometry in the service of biological assay. Ind. and Eng. Chem. Anal. Ed. 13: 84-88.
7. DOMSCH, K. H. 1958. Die wirkung von bodenfungiciden. Zeitschr. fur Pflanzk. 7: 385-405.
8. HORSFALL, J. G. 1956. Principles of Fungicidal Action. Chronica Botanica Company, Walthan, Mass.
9. KENDRICK, J. B., Jr., and JOHN T. MIDDLETON. 1954. The efficacy of certain chemicals as fungicides for a variety of fruit, root and vascular pathogens. Plant Disease Reptr. 38: 350-353.
10. KENDRICK, J. B., Jr., and G. A. ZENTMYER. 1957. Recent advances in control of soil fungi. Advances in Pest Control Research 1: 219-275, Interscience Publishers.
11. RANNEY, C. D., and L. S. BIRD, 1956. Greenhouse evaluation of in-the-furrow fungicides at two temperatures as a control measure for cotton seedling necrosis. Plant Disease Reptr. 40: 1032-1040.
12. SINCLAIR, J. B. 1957. Laboratory and greenhouse screening of various fungicides for control of Rhizoctonia damping-off of cotton seedlings. Plant Disease Reptr. 41: 1045-1050.
13. SINCLAIR, J. B. 1958. Greenhouse screening of certain fungicides for control of Rhizoctonia damping-off of cotton seedlings. Plant Disease Reptr. 42: 1084-1088.
14. STOVER, R. H. 1953. The effect of soil moisture on the growth and survival of Fusarium oxysporum f. cubense in the laboratory. Phytopathology 43: 499-504.
15. ZENTMYER, G. A. 1952. Evaluation of soil fungicides for control of Phytophthora cinnamomi. (Abst.) Phytopathology 42: 24.

OLIN MATHIESON CHEMICAL CORPORATION,
PORT JEFFERSON STATION, NEW YORK

HEAT-INDUCED STOLON ROT OF LADINO WHITE CLOVER IN THE GREENHOUSE[1]

R. A. Kilpatrick[2]

Abstract

Ladino white clover stolons were exposed to temperatures, increasing from 20° to 53° C, for 10 minutes. Stolon injury, comparable to that found in the field, was reproduced artificially. Plants placed in a moist chamber developed severe stolon rot following the initial heat injury. A complex of soil-borne fungi appear to be secondary invaders in the problem of stolon rots of Ladino white clover.

INTRODUCTION

Injury to and rotting of Ladino white clover (Trifolium repens) stolons is most common during the summer months. Garren (1) found that stolon injury increased appreciably following exposure to high temperatures. Since 1955, observations on space-planted Ladino clover in New England have indicated that stolon injury is first observed in July. In August the rotting becomes progressively worse until stolons are severed from the mother plant or killed. Tissues cultured from rotted stolons have yielded a complex of soil-borne fungi (1, 2). Bleached or lightly discolored tissues plated on agar failed to yield fungus colonies.

Symptoms vary according to time of year. During mid-summer, in New Hampshire, a bleached or blistered area is first noticeable on the stolon surface exposed to the sun. Later the bleached area turns brown and rots. The lower surface of stolons in contact with soil remains green and appears healthy in early stages. Symptoms are most commonly found on scattered plants, whereas those growing in a mixed stand seldom show this type of stolon injury. The symptoms suggest that solar radiation is the causal agent inciting the stolon injury and that fungi are of secondary importance.

This study was initiated to determine if: 1) similar stolon injury could be artificially induced with heat, and 2) rotting of stolons would develop following the initial injury.

MATERIALS AND METHODS

Clonal cuttings of Ladino white clover were transplanted to flats containing a greenhouse potting soil. Vigorously growing plants were permitted to develop stolons 8 to 10 inches long. Approximately 2 weeks before heat treatment, plants were divided into two groups. Water was withheld from one group of plants until they wilted (3 to 5 days); the other group was watered daily. Stolons were exposed to heat from an American Optical Company lamp, model 370. The lamp was set approximately 4 to 5 inches from the stolons (Fig. 1). The diameter of the light-exposed area was 2 to 3 inches. A thermometer was placed in the center of the light ray on the stolon and surface temperatures were recorded at 1-minute intervals. Stolons were exposed to progressively greater temperatures from 29° C for 1 minute to 53° for 10 minutes (Table 1). After exposure, plants were placed in a moist chamber (28°) with 100% relative humidity at night-time only. Even though desiccation of exposed areas was visible within 24 hours, final readings were not made until 13 days later. Isolations were made from rotted tissues at the time of final observation. The tests were replicated 6 to 10 times. Results were confirmed by a similar test on different clones.

RESULTS

Injury to stolons was evident 1 hour after exposure to 53° C for 10 minutes. Stolons were blistered and started to shrivel in treated areas. Three to 5 days later discoloration and rotting were evident, especially on the upper surface. Rotting progressed rapidly in all plants maintained under high humidity, whereas low humidity retarded development of diseased areas.

---

[1]Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture and Departments of Botany and Agronomy, New Hampshire Agricultural Experiment Station, Durham. Published with the approval of the Director of the New Hampshire Agricultural Experiment Station as Scientific Contribution No. 253.

[2]Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture.

FIGURE 1. American Optical Company microscope lamp used for inducing stolon injury of Ladino white clover. Note thermometer resting on the stolon.

Table 1. Average temperatures of external surface of stolons during 10-minute exposure[a].

Exposure time (in minutes)	Temperature (°C)
Initiation	29
1	38
2	43
3	46
4	49
5	50
6	51
7	52
8	52
9	53
10	53

[a]Average of 10 replications.

Wilted plants were more sensitive to heat injury than non-wilted plants. In an earlier test, severely wilted plants showed blistering following 5-minutes' continuous exposure to 33°C. In the same test, non-wilted plants required 2 hours' exposure at comparable temperature to initiate injury.

Isolation from discolored tissues was made on potato-dextrose agar. A complex of soil-borne fungi were obtained, including Fusarium spp., Rhizoctonia sp., Colletotrichum destructivum O'Gara, Trichoderma sp., and others.

The age of stolon tissue appeared to influence the time required for initial injury. Internodes nearest the growing tip of a stolon were less sensitive to heat than those 6 to 7 inches from the tip. In all cases, the survival of stolons depended on the amount of nodal rooting and the condition favoring growth of plants.

DISCUSSION

The results support the theory that solar radiation is a primary factor in deterioration of Ladino clover stolons. It is further supported by failure to obtain fungi from lightly discolored stolons in the field.

Temperatures used to induce injury were obtained in a shorter period in the greenhouse than those occurring in the field. However, soil temperatures of 30° to 40° C are not uncommon during the summer in New Hampshire.

Relative humidity also plays an important role in development of stolon rots. Under New Hampshire conditions, the maximum daily relative humidity for April to October, 1957 to 1959, varied from 96 to 100%. Since the spores and/or mycelium of the complex of soil-borne fungi are commonly found on stolons, the high humidity would favor germination and growth of the fungi, and subsequent rotting of stolons.

Literature Cited

1. GARREN, KENNETH H. 1955. Disease development and seasonal succession of pathogens of white clover. Part II. Stolon diseases and the damage-growth cycle. Plant Disease Reptr. 39: 339-341.
2. KILPATRICK, R. A., and G. M. DUNN. 1956. Late season diseases on forage crops in New Hampshire in 1955. Plant Disease Reptr. 40: 384-386.

DEPARTMENTS OF BOTANY AND AGRONOMY, UNIVERSITY OF NEW HAMPSHIRE, DURHAM

TWO SERIOUS FOLIAGE DISEASES OF WESTERN WHITE PINE IN THE INLAND EMPIRE[1]

Charles Gardner Shaw[2] and Charles D. Leaphart[3]

Abstract

Two diseases not previously differentiated are reported on western white pine from Idaho. Needle cast, caused by Hypodermella arcuata Darker, occurs primarily in the upper crown. Needle blight, with which a species of Lecanosticta is associated, is most prevalent in the lower crowns of infected trees. The sequence of development of symptoms and of the associated organisms is outlined for the two diseases.

For the past several years foresters in the Inland Empire have been increasingly concerned about discoloration of the foliage and crown deterioration of western white pine (Pinus monticola). A preliminary report on observations made during 1958 has been published (3). Since June of 1959 the authors have made repeated observations of these disorders in western white pine stands of northern Idaho. The field investigations have been supplemented by many collections of diseased foliage which have been studied microscopically. Consequently, previous observations (3) have been clarified to a considerable extent.

Two distinct diseases, not previously differentiated, are involved. They may occur as a complex on a single tree or separately on different trees. The two diseases, one primarily associated with the upper crown and the other with the lower crown, will be discussed individually as needle cast and as needle blight, respectively.

NEEDLE CAST

Symptoms: The middle and upper crowns of white pine in many pole size to mature stands show a tan brown to reddish discoloration during the growing season (Fig. 1A). The proportion of the crown involved varies considerably from tree to tree. In severely diseased trees the discolored area is somewhat conical, with the terminal leader at the apex, and involves all branches of the upper crown and portions of branches in the middle crown. If only this disease is present, the lower crown usually appears healthy.

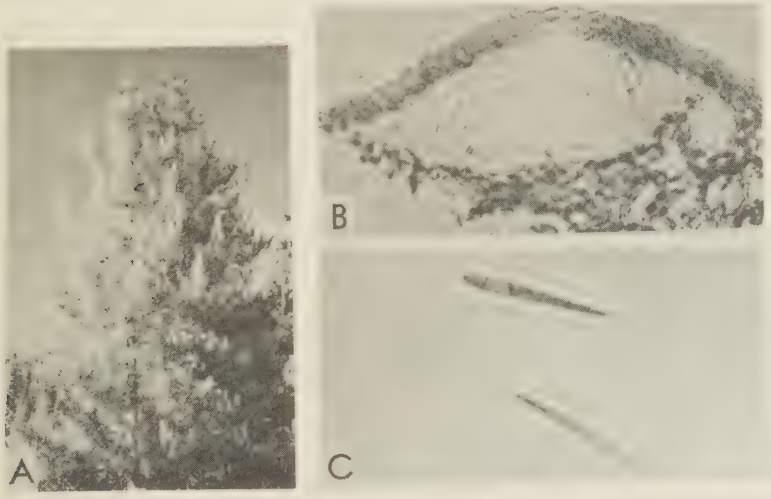

FIGURE 1. Needle cast of western white pine. A -- Upper crown of tree infected with Hypodermella arcuata Darker; healthy tree in the background. B -- Transverse section of a subhypodermal hysterothecium of H. arcuata. C -- Ascospores of H. arcuata.

[1]Scientific Paper No. 1972, Washington Agricultural Experiment Station, Pullman, Washington. Project No. 9061. The cooperation of Potlatch Forests, Inc., Lewiston, Idaho, is gratefully acknowledged.

[2]Professor and Plant Pathologist, Department of Plant Pathology, Washington State University, Pullman.

[3]Plant Pathologist, Intermountain Forest and Range Experiment Station, Forest Service, United States Department of Agriculture, Ogden, Utah; stationed at field research center, Spokane, Washington.

The intensity of discoloration in the crowns of trees showing symptoms just described is correlated with the percentage of individually discolored needles. The affected needles are always those of the previous growing season. From one to all five needles in a fascicle may be discolored. Characteristically, they are brown for their entire length.

After the discolored needles are cast in the late fall, the remaining foliage is green and apparently healthy. However, the upper crowns of badly diseased trees are thin. The branch terminals have a "lion's tail" resulting from the contrast between the full complement of current season's foliage at the branch terminal and the denudation of the remainder of the branch by the needle cast. Frequently the defoliation is so severe that only the current season's needdles remain in the upper crowns. In some white pine stands severe casting of needles has occurred for three or more successive years.

The Fungus: A needle cast fungus has been found consistently on needles showing these symptoms. The fruiting structures are present on every discolored needle and form an interrupted to almost continuous raised line along the dorsal (outer) face of the needle. The erumpent hysterothecia are concolorous to gray brown and slightly darker than the uniform light brown of the rest of the infected needle.

The hysterothecia are subhypodermal in position (Fig. 1B). The morphology of the asci and ascospores (Fig. 1C) definitely place the fungus in the genus Hypodermella. The fungus agrees in all its characters with H. arcuata Darker (2). H. arcuata previously has been reported only on sugar pine, Pinus lambertiana, from Oregon and California (2, 9). Apparently, even on this host, the fungus has been collected only occasionally.

Prevalence of the fungus in 1959, the evidence of casting in previous years, and published records of Hypodermella on this host (4, 7, 9) suggested that the fungus might have been collected previously on P. monticola. Hedgcock (4) reported H. sulcigena (Link) Tub. on P. monticola from Washington. Darker (2) considered H. sulcigena European in its distribution and consequently Hedgcock's record was transposed to H. montivaga (Petr.) Dearn. in the Host Fungus Index for the Pacific Northwest (7). Darker emphasized the similarity of H. sulcigena, H. montivaga, and H. arcuata. Therefore, collections of needle cast fungi on western white pine contained in the National Fungus Collections, United States Department of Agriculture, Beltsville, Maryland were obtained[4] on loan. No collections of H. arcuata on western white pine were found in this material.

Sequence of Development: Needle discoloration first appears in the spring of the year (April) and becomes increasingly conspicuous during May and early June. Hysterothecia are differentiated on the discolored needles during May and are apparent by mid-June, when the infected needles are approximately 13 months[5] old. Subsequent elongation of the current season's needles progressively obscures the discoloration during the growing season.

The hysterothecia mature during July and August. The time depends to some extent on the locality and the site. Water mounts of hysterothecia collected between mid-July and late August characteristically contain abundant ascospores free from the asci as well as many asci still containing ascospores. Similar mounts from hysterothecia collected in September and early October contain very few free ascospores, and the asci that can still be discerned are empty.

Casting of the needles is apparently correlated with completion of spore production and discharge. Few infected needles can be observed still attached in October. Needles bearing hysterothecia have been found on the ground as late as the first week in December. No ascospores were present in mounts made from these needles.

Needles of the current season show no signs or symptoms during the growing season or the following fall and winter. However, needle infection probably occurs between mid-July and early September when the needles are 2 to 5 months old. Bagging studies presently under way will further pinpoint the infection period.

NEEDLE BLIGHT

Symptoms: Many stands of western white pine show discoloration and deterioration primarily of the lower crown (Fig. 2A), in contrast to the symptoms of H. arcuata. Of the various descriptive names applied to this condition, needle blight of western white pine seems the most appropriate.

---

[4]The authors thank John A. Stevenson and C. R. Benjamin for making this material available.

[5]Needle ages are stated in months for precision. Although the time at which needle elongation commences varies as much as a month or more in the Inland Empire, mid-May is an approximate date for the beginning of the process.

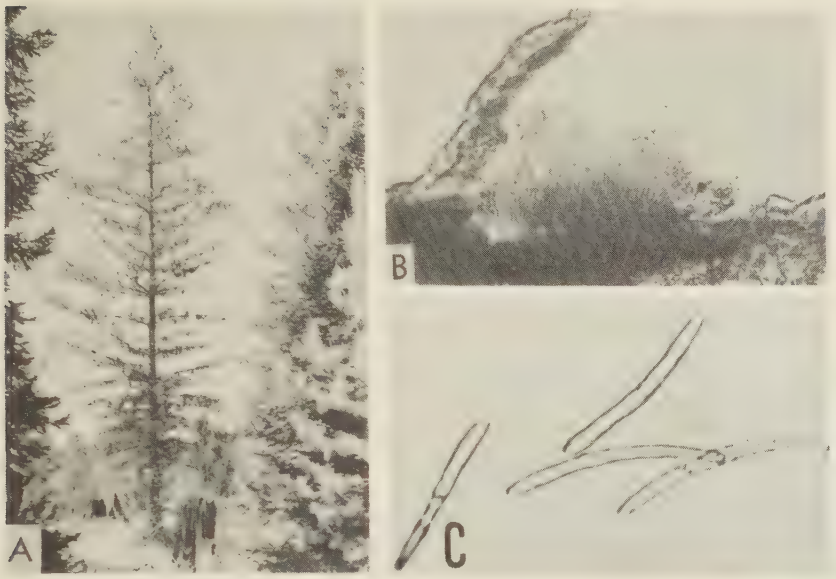

FIGURE 2. Needle blight of western white pine. A -- Pole size tree showing symptoms of severe needle blight. B -- Transverse section of stromatic fructification of Lecanosticta sp., found associated with needle blight. C -- Conidia of Lecanosticta sp.

During the summer months (July through September) the current season's foliage (needles 2 to 4 months old) is apparently healthy, except that on severely diseased trees these needles may be shorter than normal. Varying percentages of the needles from the previous growing season (needles 14 to 16 months old) on diseased trees possess reddish brown tips, involving from one-fifth to as much as one-third of the total needle length. Both the color and the proportion of the needle discolored distinguish this disease from that caused by H. arcuata. Two-year-old diseased needles (26 to 28 months old) are red-brown for their entire length and most of them are retained. Every needle belonging to this age class that is still attached is discolored. On some trees exhibiting these symptoms all of the needles of the 3-year-old class and older (38 to 40 months old or older) have been cast. On other trees all or most of these older needles, although dead, are retained and are bleached a grayish white.

From a distance diseased trees may exhibit two extremes in appearance, as well as gradations between these extremes, depending on whether the older, dead needles are retained or not. If the older gray needles have been shed, the lower crown appears thin and scrawny and shows a reddish discoloration. If retained, the lower crown appears extremely ragged and hoary gray.

Needle blight is most frequently encountered in pole size and younger stands, but is also found in mature stands. Furthermore, although the symptoms are usually restricted to the lower crown, they extend into the middle crown and even the upper crown in some trees. In these cases, however, the symptom pattern is the reverse of that caused by H. arcuata. Comparison of the two diseases gives the distinct impression that the more severe the needle cast, the farther down into the middle and lower crown it extends, while the more severe the blight, the farther up it extends into the middle and upper crown. Both the needle cast and the blight are present in varying degrees of severity in numerous areas. In such stands, both diseases are frequently present on the same trees and may mix in midcrown.

Symptom Development: During the fall, essentially no change in color occurs in trees affected by needle blight. By mid-December, however, on apparently more exposed sites, the discoloration on some 1 1/2-year-old needles (19 months) begins to extend toward the bases of the needles. Discoloration of the basal portions of diseased needles is initially lighter in color than that of the tip. During January, February, and March the progressive discoloration of the needles of this age class becomes more obvious and the color becomes a deeper reddish brown. The extension of discoloration to the bases of needles is most apparent on diseased trees in the open, on edges of stands, and on sites with exposures to the south and southwest. In more dense stands needles of this age class (22 months old in mid-March) still show tip discoloration only.

By early April these needles (now 23 months old) are discolored to their bases on practically all sites. For the first time, discoloration is also found on needles of the previous growing season (11 months old). Discoloration of the latter is initially light brown and characteristically involves only the needle tip. By June the needle tips are red brown.

The Fungus: Although several species of fungi are found on blighted needles, particularly on the older bleached ones, only one fungus has been found consistently associated with these symptoms. This fungus is usually the only one apparent on needles 11 to 13 months old on which the discoloration is restricted to the tip. As the discoloration extends down the needles, additional fructifications of this fungus appear on the newly discolored areas. The minute, elongate, black, erumpent, stromatic fructifications (Fig. 2B) are just discernible to the naked eye.

The close association of this fungus with the symptoms described was further indicated by placing diseased branches collected in January and March in water in Erlenmeyer flasks in the greenhouse. When collected, the needles of the previous growing season (8 to 10 months old) showed no symptoms. After 23 days for specimens collected in January and 15 days for specimens collected in early March, the tips of some of the needles of the previous growing season had discolored. The stromata of this fungus were present on the discolored tips; no other fungi were seen. The symptoms and fructifications developed as a result of forcing were comparable in every respect with those that developed later (in April) on needles of the same age class in diseased stands.

Comparison of the fungus with similar organisms reported on the needles of Pinus spp. (5, 8) indicates that it is similar to Lecanosticta acicola (Thuem.) Syd. (8, 10). Specimens were sent to Paul V. Siggers (8) and John S. Boyce, Jr. (1) for examination. Both concurred in the tentative identification of the fungus as a species of Lecanosticta.

A description of the fungus follows: Stromata black, ellipsoid to elliptic in outline, 0.3-1.3 x 0.15-0.3 mm, oriented parallel to the longitudinal axis of the needle, subhypodermal in origin, becoming erumpent by an elongate slit and lifting the host hypodermis and epidermis, which remain intact and laterally attached as a flap, in cross section appearing blackish brown at the base and fading to light yellow brown above, 80-140μ in height, plectenchymatous, composed of upright, septate conidiophores; conidiophores darker at the base and composed of shorter, stouter cells basally, lighter in color and thinner above; conidia produced apically on the conidiophores and superficially on the stroma, agglutinated and dispersing slowly in water, at first hyaline, becoming light yellow-brown, scolecosporous, continuous to 3-5 septate, curved or rarely straight, variable in length, 35-66 x 3.0-4.2μ and with the ends attenuate rounded in some collections, 23-43 x 3.5-4.2μ and apically rounded and basally bluntly truncate in other collections (Fig. 2C).

Sporulating stromata of this fungus have been found on needles from 10 to 28 months of age. Sporulation is more profuse on needles 10 to 15 months old, and becomes sparser on older needles. Although the stromata are conspicuous on bleached, 36-months-old and older needles no sporulation has been found on them.

The spores of the fungus on western white pine (Fig. 2C) do not become darker than yellow brown, while those of L. acicola become olive brown to "smoky" when mature. The shorter, stouter spores found in some collections on western white pine agree in size and shape with those of L. acicola better than do the longer spores. The stromata of the western fungus are more robust and of greater height than those encountered with the Lecanosticta stage of Scirrhia acicola (Dearn.) Siggers. Repeated attempts to find an ascigerous stage associated with the stromata of our fungus have been unsuccessful. For these reasons, the fungus is assigned to the genus Lecanosticta, but it is not referred to L. acicola.

A collection from Pinus strobus planted in Montana agrees in all details with those from Pinus monticola. The Montana collection has particular interest in view of the recent report by Boyce (1) of L. acicola on eastern white pine in North Carolina. Furthermore, the symptoms reported by Boyce are similar to those observed on Pinus monticola and P. strobus in the West.

In addition to the fungus identified as Lecanosticta sp., several other fungi have been encountered frequently but not consistently on blighted foliage. Study of these organisms, and of the Lecanosticta as well, is continuing.

One species of aphid belonging to the genus Pineus (3) also has been found frequently in association with needle blight of western white pine. The significance of the aphid in the blight complex remains to be determined. But the sequence of symptom development reported herein would seem to exclude the aphid as the primary factor responsible for needle blight.

Denton and Leaphart (3) also reported the occurrence of cankers on the 1956 and older twig growth. While old cankers are still discernible, new cankers have not developed either on the older growth or on twig growth of subsequent years. Consequently, there seems to be no correlation of the cankers with the blighting of the needles during the past 3 years. Molnar and Silver (6) have recently reported the association of Aureobasidium pullulans (d By.) Arnaud (=Pullularia pullulans (d By.) Berk.) with similar cankers on western white pine.

Literature Cited

1. BOYCE, J. S., Jr. 1959. Brown spot needle blight on eastern white pine. Plant Disease Reptr. 43: 420.
2. DARKER, G. D. 1932. The Hypodermataceae of conifers. Contrib. Arnold Arbor. I. 131 pp.
3. DENTON, R. E., and C. D. LEAPHART. 1959. Symptoms of abnormal crown deterioration in western white pine stands. Intermount. Forest and Range Exp. Sta. Research Note 69. 4 pp.
4. HEDGCOCK, G. G. 1932. Notes on the distribution of some fungi associated with diseases of conifers. Plant Disease Reptr. 16: 28-42.
5. HULBARY, R. L. 1941. A needle blight of Austrian pine. Illinois Nat. Hist. Survey Bull. 21: 231-236.
6. MOLNAR, A. C., and G. T. SILVER. 1959. Build-up of Pullularia pullulans (deBary) Berkhout within a severe spruce budworm infestation at Babine Lake, British Columbia. The Forestry Chronicle 35: 227-231.
7. SHAW, C. G. 1958. Host fungus index for the Pacific Northwest. I. Hosts. Wash. Agr. Exp. Sta. Circ. 335. 127 pp.
8. SIGGERS, P. V. 1944. The brown spot needle blight of pine seedlings. U. S. Dept. Agr. Tech. Bull. 870. 36 pp.
9. WEISS, F., and MURIEL J. O'BRIEN. 1952. Index of plant diseases in the United States. Plant Disease Survey Spec. Publ. 1(4): 530-806.
10. WOLF, F. A., and W. J. BARBOUR. 1941. Brown-spot needle disease of pines. Phytopathology 31: 61-74.

WASHINGTON AGRICULTURAL EXPERIMENT STATION, PULLMAN AND INTERMOUNTAIN FOREST AND RANGE EXPERIMENT STATION, FOREST SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, OGDEN, UTAH

A PRELIMINARY REPORT ON THE USE OF PIMARICIN IN THE ISOLATION OF PHYTOPHTHORA SPP. FROM ROOT TISSUES[1]

J. W. Eckert and Peter H. Tsao

Rapid development of secondary fungi and bacteria often limits the usefulness of agar media for isolation of Phytophthora species from root tissues. A simple means of suppressing growth of these contaminants, without adversely affecting the development of Phytophthora species, could be advantageously used for both routine diagnosis and surveys involving root rot problems.

Published antifungal spectra of two of the tetraene antibiotics, nystatin (2, 3) and pimaricin (3, 4), suggested that these materials might be useful in the formulation of selective media for the isolation of pythiaceous fungi. Results of preliminary tests with these antibiotics are reported below.

IN VITRO EVALUATIONS

Neither nystatin[2] (2990 units/mg) nor pimaricin[3] (Na salt, 820 µg/mg) at 100 ppm in corn meal agar (Difco) inhibited growth or sporulation of Phytophthora cactorum, P. capsici, P. cinnamomi, P. citrophthora, or P. parasitica. Eighteen species (representing 13 genera) of mucors and imperfect fungi, all common soil fungi and often associated with root platings, were completely inhibited for 6 or more days by either of the antibiotics.

ISOLATIONS FROM INFECTED ROOT PIECES

Sodium pimaricin was the antibiotic of choice for root plating experiments because its relatively high water solubility facilitated routine preparation of the selective medium. Citrus fibrous root pieces (about 1 cm in length) suspected of Phytophthora infection were pooled, surface sterilized for 1 to 1.5 minutes in 0.5% sodium hypochlorite (10% Clorox), rinsed three times in sterile distilled water, and randomly plated with equal numbers on corn meal agar medium (control) and on the same medium containing 100 ppm sodium pimaricin, 50 ppm sodium penicillin G (1650 units/mg), and 50 ppm polymyxin B sulfate (7760 units/mg). The latter two antibiotics were also noninhibitory against the Phytophthora spp. tested and served to suppress bacterial contaminants. Details of the development of this selective medium have been reported elsewhere (1).

Table 1. Recoveries of Phytophthora, Pythium, and other fungi from fibrous roots on selective antibiotic agar medium versus corn meal agar medium.

Experiment number	Number of root pieces[b] per medium	Corn meal agar (control)				Corn meal agar + pimaricin[a]			
		Phytophthora		Pythium spp.	Other fungi	Phytophthora		Pythium spp.	Other fungi[c]
		parasitica	citrophthora			parasitica	citrophthora		
1	75	0	0	21	73	2	3	17	0
2	50	4	-	-	49	11	-	-	0
3	50	-	-	38	50	-	-	41	0
4	50	0	-	33	50	7	-	37	0
5	50	0	-	27	49	14	-	24	0
6	50	0	-	-	42	2	-	-	0
7	50	1	0	13	47	1	1	25	0
8	50	-	1	5	50	-	21	8	0

[a] 50 ppm penicillin and 50 ppm polymyxin added to suppress bacteria.

[b] All roots taken from soils naturally infested with Phytophthora spp., except in the 2nd and 8th experiments, from natural soils artificially infested with P. parasitica and P. citrophthora, respectively.

[c] Species of Mucorales, Fusarium, Trichoderma, Penicillium, Gliocladium, Torula, Pyrenochaeta, Alternaria, and so forth.

[1] The authors wish to express their appreciation to Dr. L. J. Klotz for samples of pimaricin and to Miss Karla B. Jones for technical assistance in evaluating specificity of the antibiotics.

[2] Nystatin (mycostatin) generously supplied by Olin Mathieson Chemical Corp.

[3] Distributed by the Royal Netherlands Fermentation Ind., Ltd., Delft, Holland.

In eight independent experiments, as shown in Table 1, recoveries of Phytophthora citrophthora and P. parasitica were improved by the use of the antibiotics in the plating medium. Pythium species occurred with almost equal frequency on both control and antibiotic media. The most significant observation, however, was the almost complete absence of contaminating fungi in the pimaricin cultures, while at the same time there was no indication that the variety or frequency of Phytophthora or Pythium species recovered had been altered by the presence of the antibiotics. Pure culture isolation of Phytophthora or Pythium species was greatly facilitated by the selective medium.

Preliminary evaluations of the pimaricin-penicillin-polymyxin medium for isolation of Phytophthora cinnamomi from avocado roots and Phytophthora cryptogea from alfalfa roots have appeared promising[4].

Literature Cited

1. ECKERT, J. W., PETER H. TSAO, L. J. KLOTZ, and KARLA B. JONES. 1960. Use of pimaricin, penicillin, and polymyxin in the isolation of Phytophthora spp. from plant tissue. Proc. Am. Phytopath. Soc. Pacific Div. Meetings, June 15, 1960. (Abst.) Phytopathology (In press).
2. FRANK, ELEANORA, F. E. PANSY, and J. F. PAGANO. 1959. Nystatin in the control of fungal infections of orchids. Antibiotics Annual 1958-59: 898-902.
3. KLIS, J. B., L. D. WITTER, and Z. J. ORDAL. 1959. The effect of several antifungal antibiotics on the growth of common food spoilage fungi. Food Technology 13: 124-128.
4. STRUYK, A. P., I. HOETTE, G. DROST, J. M. WAISVISZ, T. van EEK, and J. C. HOOGERHEIDE. 1958. Pimaricin, a new antifungal antibiotic. Antibiotics Annual 1957-58: 878-885.

DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF CALIFORNIA, RIVERSIDE

[4]Communications with Dr. G. A. Zentmyer and Dr. D. C. Erwin, respectively, both of the University of California, Riverside.

HALO SCAB--RESULT OF AN ASSOCIATION OF ELSINOË FAWCETTI AND BREVIPALPUS PHOENICIS DESTRUCTIVE TO SOUR-ORANGE SEEDLINGS[1]

L. C. Knorr and Gino Malaguti[2]

In a citrus nursery at Maracay, Estado Aragua, Venezuela, an appreciable amount of defoliation and dying of plants was encountered recently in a planting of sour-orange seedlings. Forty % of the seedlings were variously afflicted, with symptoms ranging from a spotting of leaves to the death of plants. As in many sour-orange plantings, scab (caused by Elsinoë fawcetti Bitanc. & Jenkins) was to be seen in the foliage of nearly every plant. By themselves, scab lesions cause neither defoliation nor death of plants. But when scab lesions were found surrounded by yellow halos, 2 to 5 mm in width (Fig. 1), plants bearing such leaves were discovered to be in various stages of defoliation leading ultimately to the death of plants.

FIGURE 1. Halo scab on leaves of sour orange. Scabs are the result of Elsinoë fawcetti, and halos the work of Brevipalpus phoenicis; together these organisms cause a shedding of leaves which results in the dying of affected plants. (Photograph courtesy of Facultad de Agronomia, Universidad Central de Venezuela.)

[1]Florida Agricultural Experiment Stations Journal Series, No. 1096.

[2]Respectively, Plant Pathologist, Citrus Experiment Station, University of Florida, Lake Alfred, Florida; and Ingeniero Agronomo, Ministerio de Agricultura y Cria, Centro de Investigaciones Agronomicas, Maracay, Venezuela.

The writers wish to acknowledge the financial assistance of the Fundacion Creole, Caracas, Venezuela, which organization made possible an interchange between members of the University of Florida and the Universidad Central de Venezuela.

Microscopic examination of leaves with haloed scabs showed them to differ from leaves without halo-enclosed scabs in that enormous numbers of Brevipalpus mites thronged within the chlorotic borders, whereas in leaves without halos, few to no mites were to be seen. At times, populations were so great as to give halos and associated scabs a pinkish cast. Apparently scabs served as anchorages where nymphs and adults derived a certain amount of protection and where females found it appropriate to lay their eggs.

Brevipalpus mites, collected from affected plants in various parts of the block, were sent to Dr. Edward W. Baker, Smithsonian Institution, Washington, for identification. Examination showed that all collections consisted of the false spider mite, Brevipalpus phoenicis (G.).

Though B. phoenicis is ubiquitous -- having been found on many hosts in various parts of the world (4) -- little is known about its effect on citrus. In Florida fruits on unsprayed trees sometimes show very high populations (up to 1162 Brevipalpus mites have been counted on a single fruit), while adjacent leaves and twigs reveal but few straggling individuals. Under such circumstances fruits are usually russetted (although it remains uncertain whether B. phoenicis causes russetting), while leaves and twigs remain symptomless. Only rarely are cases encountered in which the foliage is either sufficiently attractive or mites are sufficiently numerous to result in spotting of the leaves (2). When leaf spotting does occur it resembles closely that which is produced under experimental conditions when large numbers of B. phoenicis are caged on young sweet-orange seedlings. Except for the absence of scab lesions, the spots and consequent leaf drop that develop in caged cultures resemble those found in the halo-affected foliage of the above-mentioned sour-orange seedlings.

Unchecked, various species of Brevipalpus mites on citrus may develop prodigious powers for destruction. A striking example is the devastation wrought in certain areas of Argentina and Paraguay where B. obovatus Donn., the cause of lepra explosiva, has put an end to the growing of citrus. Another species, B. californicus (Banks), produced great consternation and the passing of quarantine laws in Florida during the first three decades of the 20th Century; at the time, the troubles in question, namely nailhead rust and Florida scaly bark, were believed to be caused by the fungus Cladosporium herbarum var. citricolum (2).

Destructive as Brevipalpus mites may at times become, they are remarkably simple to control. A single application of wettable sulfur, 5 to 10 pounds in 100 gallons of water, will virtually eradicate the various members of this genus (1, 3, 5).

Literature Cited

1. BAPTIST, B. A., and D. J. W. RANAWEERA. 1955. The scarlet mites of the genus Brevipalpus as pests of tea in Ceylon. Tea Quarterly 26: 127-137.
2. KNORR, L. C. 1959. Presenting Brevipalpus mites and some questions that they pose. Citrus Magazine 21(10): 8-12, 20, 22.
3. KNORR, L. C., and W. L. THOMPSON. 1954. Spraying trials for the control of Florida scaly bark in citrus. Plant Disease Reptr. 38: 143-146.
4. PRITCHARD, A. E. and E. W. BAKER. 1952. The false spider mites of California (Acarina: Phytoptipalpidae). University of California Publ. in Ent. 9: 1-94.
5. SHERMAN, M. and M. TAMACHIRO. 1959. Toxicity of insecticides and acaricides to the papaya, Carica papaya L. Hawaii Agr. Exp. Sta. Tech. Bull. 40: 56 pp.

FLORIDA AGRICULTURAL EXPERIMENT STATION, GAINESVILLE

BOTRYTIS CINEREA AND SCLEROTINIA SCLEROTIORUM IN SEED OF SAFFLOWER AND SUNFLOWER[1]

W. E. Sackston[2]

Abstract

Botrytis cinerea Fr. and Sclerotinia sclerotiorum (Lib.) d By. were isolated from surface-sterilized seeds of safflower (Carthamus tinctorius) and sunflower (Helianthus annus) produced at various locations in Canada in different years. Botrytis has not previously been reported as an internally-borne pathogen of sunflower seed, nor Sclerotinia from safflower seed.

As both fungi cause head rots of safflower and sunflower, the seeds probably are infected by the spread of mycelium from diseased tissues of the capitulum. Dissemination of the pathogens by seed may be reduced by sowing only seed from fields free of head rot, and by treating the seed with appropriate organic mercury compounds.

INTRODUCTION

Botrytis cinerea Fr. is an ubiquitous fungus which, although not an aggressive pathogen, attacks many species of plants under favorable conditions. It has been isolated from seeds of many crop plants, but Noble et al. (7) do not record it on seeds of safflower (Carthamus tinctorius) or sunflower (Helianthus annuus) in their recent extensive list.

Sclerotinia sclerotiorum (Lib.) d By. is an aggressive pathogen on many plant species throughout the world. It has been reported on the seeds of various crop plants, in almost all cases as sclerotia mixed with the seed (7).

Studies at Winnipeg have shown that both B. cinerea and S. sclerotiorum occur in seed of safflower and sunflower. The results of those studies are presented in this paper.

MATERIALS AND METHODS

Safflower seeds studied included samples produced at Lethbridge, Alberta, in 1951, 1952, 1953, and 1954; at Foremost, Alberta, in 1952; at Winnipeg, Manitoba, in 1952 and 1954; and at Ottawa, Ontario, in 1953.

Viability of the seeds and incidence of seed-borne organisms were determined by surface-sterilizing seeds in 2% hypochlorite solution and plating them on potato-sucrose agar in Petri dishes. Some samples were also plated without surface sterilization, and after treatment with ethyl mercury p-toluene sulfonanilide (Ceresan M) or zinc ethylene bisdithiocarbamate (zineb), materials used by Thomas (12) to control seed-borne pathogens of safflower. In other tests untreated and treated seeds were sown in soil in the greenhouse, and their percentage emergence was determined.

Observations were made on sunflower seeds in a study on the effects of chemical treatments and storage for long periods (10), and on occasional samples referred for examination from various sources.

The chemicals used at various times for seed treatment included: Phenyl mercury acetate and ethyl mercury chloride (Agrox C), tetramethyl thiuram disulfide (thiram-50%), ethyl mercury p-toluene sulfonanilide (Ceresan M), phenyl mercury urea (Leytosan), ethyl mercury thioruea derivative (Lunasan), lindane with phenyl mercury acetate and ethyl mercury chloride (Mergamma C), zinc ethylene bisdithiocarbamate (zineb), and dichloronaphthoquinone (dichlone).

RESULTS

Safflower seeds produced at several locations in different years varied greatly in percentage germination and in the number and kind of seed-borne microflora. Differences between varieties were also striking. The results of some of the platings are presented in Table 1.

The high incidence of Botrytis and Sclerotinia in seed grown at Lethbridge in 1952 is particularly interesting, as both organisms are known to cause head rot of safflower in Canada

[1]Contribution No. 60 from Canada Department of Agriculture Research Station, Winnipeg, Manitoba.

[2]Head, Plant Pathology Laboratory.

Table 1. Germination and microflora of safflower seed surface-sterilized and plated on agar.

Year	Seed source: Location	Variety	% germination	% clean seed	% bacteria	% miscellaneous fungi[a]	% Botrytis	% Sclerotinia
1951	Lethbridge	N-6	79	92	2	4	0	0
1952	Foremost	N-8	22	42	36	16	1	1
1952	Lethbridge	N-3	2	0	66	72	6	26[b]
		N-6	0	0	8	6	38	34
		N-8	36	40	6	40	0	20
		N-6xN-8	0	0	60	56	18	10
		N-10	4	0	10	24	32	74
		N-852	14	0	0	44	14	56
		Indian	72	22	28	42	0	28
1952	Winnipeg	N-6	10	41	11	52	1	0
		N-8	6	13	72	26	0	0
		N-9	1	27	40	40	1	0
		N-10	12	23	36	47	0	0
		N-852	6	35	17	48	0	0
		CVO-308	3	19	29	63	1	0
		CVO-852	11	27	20	56	0	0
		Indian	9	28	49	28	0	0
1953	Lethbridge	N-3	94	92	0	6	2	2
	Dry-land plots	N-6	70	72	0	20	2	0
		N-8	94	92	0	8	2	2
		N-10	76	30	0	56	6	0
		N-520	80	68	2	20	4	2
		N-852	92	42	0	48	2	0
		Indian	89	90	0	10	0	0
	Irrigated plots	N-6	42	76	8	22	6	2
		N-8	18	50	12	10	6	1
		N-520	58	82	6	12	6	1
		Indian	26	62	0	2	4	0
1953	Ottawa	N-852	18	1	3	99	0	0
1954	Lethbridge dry land	N-6xN-8	11	14	19	64	6	1
		N-8	15	12	41	44	7	0
		N-10	9	1	--	75	14	0
		N-852	52	0	--	74	26	3
		WO-14	14	2	23	77	14	0
		2377	10	4	--	90	15	0
		3614	21	13	32	56	8	0
		4035	17	10	25	69	6	0
		Indian	5	1	40	90	19	1
1954	Winnipeg	N-852	1	0	46	97	1	0
		WO-14	3	0	69	88	0	0
		RL-1013	2	0	76	74	0	0
		RL-1014	22	0	54	83	0	0
		CD-5708	2	0	63	100	0	0
		CD-5766	1	0	9	100	0	0
		Sask.-5269	25	0	62	90	0	0
		Indian	24	0	56	94	0	0

[a]Miscellaneous fungi include species of: Alternaria, Aspergillus, Cladosporium, Epicoccum, Fusarium, Helminthosporium, Penicillium, Rhizopus, Trichoderma, and unidentified colonies.
[b]The very high incidence of Sclerotinia in some seed lots produced at Lethbridge in 1952 was due to difficulty in distinguishing individual colonies of the fungus in some plates in which many seeds were overrun.

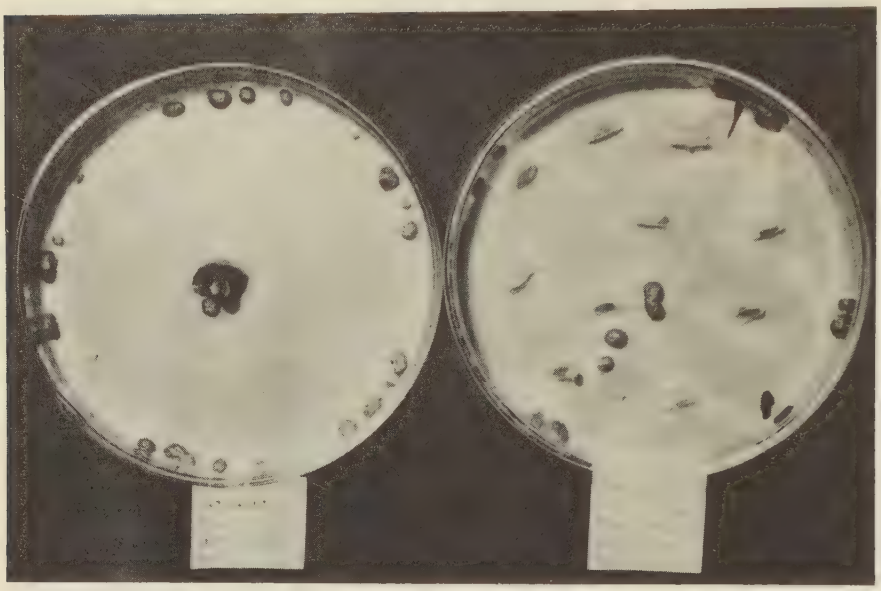

FIGURE 1. Sclerotinia sclerotiorum growing from a sclerotium and from clean seeds taken from a safflower head with Sclerotinia head rot.

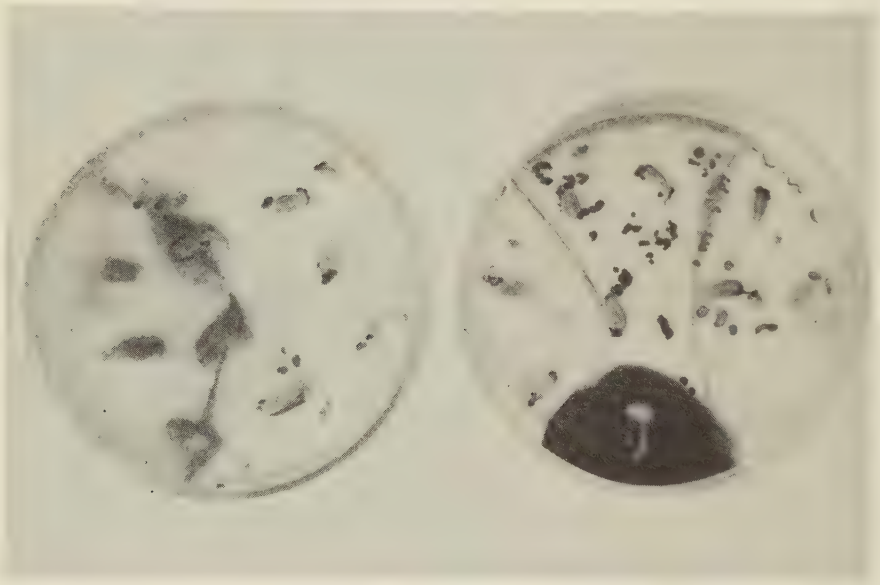

FIGURE 2. Botrytis cinerea growing from safflower seeds; profusely sporulating colonies and some sclerotial colonies in plate on left, largely sclerotial colonies in plate on right.

(2, 3, 4, 8, 13), as well as in other countries (1). Vanterpool (13) isolated B. cinerea from three partially developed seeds from a safflower head infected by S. sclerotiorum in Saskatchewan. Pure cultures of S. sclerotiorum were isolated from apparently healthy seeds taken from safflower heads affected by Sclerotinia head rot in Manitoba (Fig. 1).

Some colonies of Botrytis on the Lethbridge safflower seeds sporulated profusely; others formed numerous irregular sclerotia, but produced few spores (Fig. 2). Cultures of both types submitted to Dr. J. Walton Groves at Ottawa were assigned to B. cinerea.

Seeds of the variety N-6, produced at Lethbridge in 1952, were surface-sterilized in hypochlorite solution for various intervals and plated. Sterilization for 5 minutes did not appreciably reduce the incidence of B. cinerea or of Alternaria tenuis Nees, which was present on most seeds. Both fungi must penetrate relatively deeply into the tissues of infected seeds to survive such severe surface sterilization.

Seeds of each of the seven varieties grown at Lethbridge in 1952 were treated with Ceresan M and Zineb respectively, and plated on agar. Treatment with zineb gave results comparable

to light surface sterilization with hypochlorite solution; it was not nearly so effective as Ceresan, which gave almost complete control of Botrytis, Sclerotinia, and miscellaneous fungi.

Seeds of N-8 and N-852 grown at Lethbridge in 1952 were sown in soil in the greenhouse at Winnipeg without treatment, and after treatment with Ceresan M and zineb respectively. The germination of untreated seed of N-8 was 20%, and of N-852 was 9%. Seed of the two varieties treated with Ceresan germinated 53% and 26% respectively. Treatment with zineb was less effective. Much less striking results were obtained with Ceresan treatment of seed produced at Lethbridge in 1954. Average emergence of untreated seed of nine varieties was 21%, of Ceresan-treated seed was 24%. Ceresan treatment of seed of eight varieties produced at Winnipeg in 1952 depressed emergence from an average of 38% to 18% for the treated seed.

Sunflower seeds plated on agar at Winnipeg have occasionally yielded B. cinerea. Most of the isolations were made from seeds grown in Manitoba in the period 1951 to 1959, but Botrytis was also isolated from three samples produced in Alberta in 1959. Some of the sunflower seeds which yielded Botrytis colonies had been treated with thiram-50%, Agrox C, dichlone, or hydroxymercurichlorophenol 3.2%, hydroxymercurinitrophenol 12% (Semesan Bel) (10). Some of the samples surface-sterilized with hypochlorite solution and plated yielded Botrytis from a trace to 5% of the seeds. Both sporulating and sclerotial type colonies were produced.

In one sample of sunflower seeds plated without surface sterilization more than 30% of the seeds gave rise to Botrytis. When unsterilized seeds of the same lot were dehulled before plating, about 10% of the kernels yielded Botrytis colonies. Dissection of infested seeds indicated that the Botrytis was confined almost entirely to the hull and the testa.

Seed-borne Botrytis caused a seedling blight of sunflowers grown from seed which had been treated with Agrox C, thiram-50%, Leytosan, Lunasan, or Mergamma C (10). Although the number of seedlings affected was small, the occurrence was interesting because Botrytis had not been reported previously as a seed-borne pathogen of sunflowers.

S. sclerotiorum mycelium was found adhering to sunflower seeds by Mujica (6) in Chile. Young and Morris (14) in the United States and Lobik (5) in the U.S.S.R. found sclerotia of S. sclerotiorum on and in sunflower seeds. Sunflower seeds taken from heads rotted by Sclerotinia have frequently given rise to S. sclerotiorum on agar at Winnipeg. However, S. sclerotiorum has been isolated at Winnipeg from samples of apparently healthy sunflower seeds only four times in 9 years, in each case from only one or two seeds in the sample.

DISCUSSION

Sunflower growing was confined almost entirely to southern Manitoba until 1958, when commercial production started in southern Alberta. Safflowers were grown in Western Canada on an experimental basis until contract growing began in southern Alberta in 1957. Head rots induced by B. cinerea and S. sclerotiorum, although present in most years, have caused very little loss in sunflowers in Manitoba (9), and have been of only academic interest on safflowers. The situation may change radically with large scale production of both crops in southern Alberta, particularly if they are grown under irrigation. If head rot caused by Botrytis or Sclerotinia should become an important disease of either sunflower or safflower in Alberta, incidence of the pathogens in seed of these crops could be expected to increase greatly.

The germination of safflower seed is erratic and hard to predict. It appears to be influenced adversely by dull, cool weather at flowering time and later. These conditions also favor the development of head rots caused by Botrytis and Sclerotinia. Seed germination may be poor even in the absence of these pathogens, as shown in the results reported here. Seeds of both safflower and sunflower infected by Botrytis may germinate reasonably well. The resulting seedlings are usually killed rapidly, however, if they are produced in Petri dishes or in soil maintained at high moisture levels. If the moisture is restricted the seedlings may produce healthy plants, as observed for safflower by Savile (11). Seeds attacked by Sclerotinia are usually killed. Whether alive or dead, infected seeds may serve as foci of infection for Botrytis seedling blight, or to introduce Sclerotinia into soil previously free of infestation.

Seed infection by Sclerotinia appears to be relatively rare except in samples taken from diseased heads. Dissemination of Sclerotinia by seed of safflower and sunflower may therefore be reduced by sowing only seed known to come from fields free of head rot. It may prove necessary in Alberta to grow crops for seed purposes in dry land areas without irrigation. As a further precaution, seed should be treated before sowing with an organic mercury fungicide; although such treatment may not give complete control of seed-borne Botrytis and Sclerotinia, it will minimize their spread.

ACKNOWLEDGMENTS

The author is grateful for the samples of safflower and sunflower seeds so generously supplied by individuals at the Experimental Farms at Lethbridge, Morden, and Ottawa, at Co-operative Vegetable Oils Ltd., Altona, Manitoba, and in the Plant Science Department, University of Manitoba, Winnipeg.

Literature Cited

1. BOHOVIK, I. V. 1936. Diseases of the newly introduced oleiferous crops in the Ukraine. (Collection of scientific papers on plant protection): 114-123. Ukr. State Publ. Office Collect. Co-op. Fmg.-Lit., Kiev, 1936. (In Rev. Appl. Mycol. 16: 750. 1937).
2. ELLIOTT, MARY E. 1954. Gray mould of safflower. In Report of Can. Plant Disease Survey 34: 44.
3. HARPER, F. R. 1952. Head blight of safflower. In Report of Can. Plant Disease Survey 32: 34.
4. HARPER, F. R. 1954. Head blight of safflower. In Report of Can. Plant Disease Survey 34: 44.
5. LOBIK, A. I. 1928. Wholesale destruction of sunflower caused by Sclerotinia libertiana. Proc. Pan-Soviet Congress of Botanists in Leningrad in January 1928: 177-178. (In Rev. Appl. Mycol. 9: 458. 1930).
6. MUJICA, FERNANDO R. 1950. Medidas adicionales de control de la esclerotiniosis del girasol. Agric.-tec., Chile, 10(2): 74-78.
7. NOBLE, MARY, J. de TEMPE, and PAUL NEERGAARD. 1958. An annotated list of seed-borne diseases. Commonwealth Mycol. Inst., Kew, and Int. Seed Test. Assoc.
8. SACKSTON, W. E. 1953. Head blight of safflower. In Report of Can. Plant Disease Survey 33: 41.
9. SACKSTON, W. E. Sunflower diseases in Manitoba. In Reports of Can. Plant Disease Survey 1948 to 1959.
10. SACKSTON, W. E., and BARBARA M. CHERNICK. Effects of chemical treatment and storage on sunflower seeds. Can. J. Plant Sci. (In press).
11. SAVILE, D. B. O. 1943. Seedling blight of safflower. In Report of Can. Plant Disease Survey 23: 28.
12. THOMAS, C. A. 1952. Transmission of safflower rust on treated seed. Phytopathology 42: 108-109.
13. VANTERPOOL, T. C. 1951. Head rot of safflower. In Report of Can. Plant Disease Survey 31: 32.
14. YOUNG, P. A., and H. E. MORRIS. 1927. Sclerotinia wilt of sunflowers. Montana Agr. Exp. Sta. Bull. 208. 32 pp.

RESEARCH BRANCH, CANADA DEPARTMENT OF AGRICULTURE, WINNIPEG, MANITOBA

EPIDEMIC LEAF-BLIGHTING OF CALIFORNIA-LAUREL

J. R. Parmeter, Jr.[1], R. V. Bega[2], and J. R. Hood[3]

Summary

Extensive blighting of the leaves of California-laurel, Umbellularia californica, occurred during the winter of 1957-58 in the central coastal region of California. Pseudomonas lauracearum, Kabatiella phoradendri f. umbellulariae, and Colletotrichum gloeosporioides were associated with the blight. Subsequent branch dieback associated with Botryosphaeria sp. developed during the following summer. Inoculation experiments demonstrated the pathogenicity of C. gloeosporioides on California-laurel. Examination of weather records suggested that the sudden epidemic was a result of the unusually heavy precipitation during the winters of 1955-56 and 1957-58.

California-laurel, Umbellularia californica, is an important tree species in the coastal mountains of California. In addition to its esthetic value in native stands, it is used widely in home and park landscaping. During the winter of 1957-58, laurels throughout the central coastal region of California were affected with severe leaf blighting. Since such epidemic damage to laurel had not previously been reported, investigation of the cause was undertaken.

SYMPTOMS

Affected leaves showed few to many angular, black spots (Fig. 1) resembling those associated with Pseudomonas lauracearum Harvey (2). In addition most leaves showed irregular, brown, necrotic areas involving varying amounts of the leaf area (Fig. 2).

The severity of blighting on individual trees was variable. At lower elevations severe leaf-blight was restricted to the lower portion of the crown. At higher elevations the entire crown of most trees was severely damaged, giving the trees a uniform, brown or scorched appearance. The trees remained alive, however, and new shoots were produced in the spring. The amount of new foliage depended on the severity of the blighting. Trees that were heavily damaged produced sparse foliage, and numerous dead twigs were evident. Lightly affected trees produced a normal crop of leaves.

On many trees a portion of the new leaves turned black and the tips of new shoots died. Damage was not great, since only a few shoots on any one tree were so affected. During the summer of 1958, additional dieback involving branches as large as 1 inch in diameter developed. These dead branches were scattered through the crown of many trees, the yellow leaves standing out in marked contrast to the green crown.

CAUSAL ORGANISMS

Diseased leaves incubated in moist chambers yielded three pathogens. P. lauracearum was isolated from bacterial ooze exuded from the black, angular spots. Kabatiella phoradendri (Darling) Harvey f. umbellulariae Harvey and Colletotrichum gloeosporioides Penz. were isolated from acervuli produced on the brown, necrotic areas. C. gloeosporioides was prevalent on all such lesions. K. phoradendri was isolated less frequently. C. gloeosporioides was also the only organism consistently associated with the black leaf and twig dieback of new shoots.

When sections of branches showing dieback during the summer were incubated in moist chambers, numerous perithecia of Botryosphaeria were produced along with pycnidia of Dothiorella and Botryodiplodia imperfect stages. The occurrence of both imperfect types suggested that two forms of Botryosphaeria were associated with the dieback. No attempt was made to determine species.

[1]Assistant Professor of Plant Pathology, Department of Plant Pathology, University of California, Berkeley, California.
[2]Plant Pathologist, Division of Forest Disease Research, Pacific Southwest Forest and Range Experiment Station, United States Department of Agriculture, Berkeley, California.
[3]Laboratory Technician II, Department of Plant Pathology, University of California, Berkeley, California.

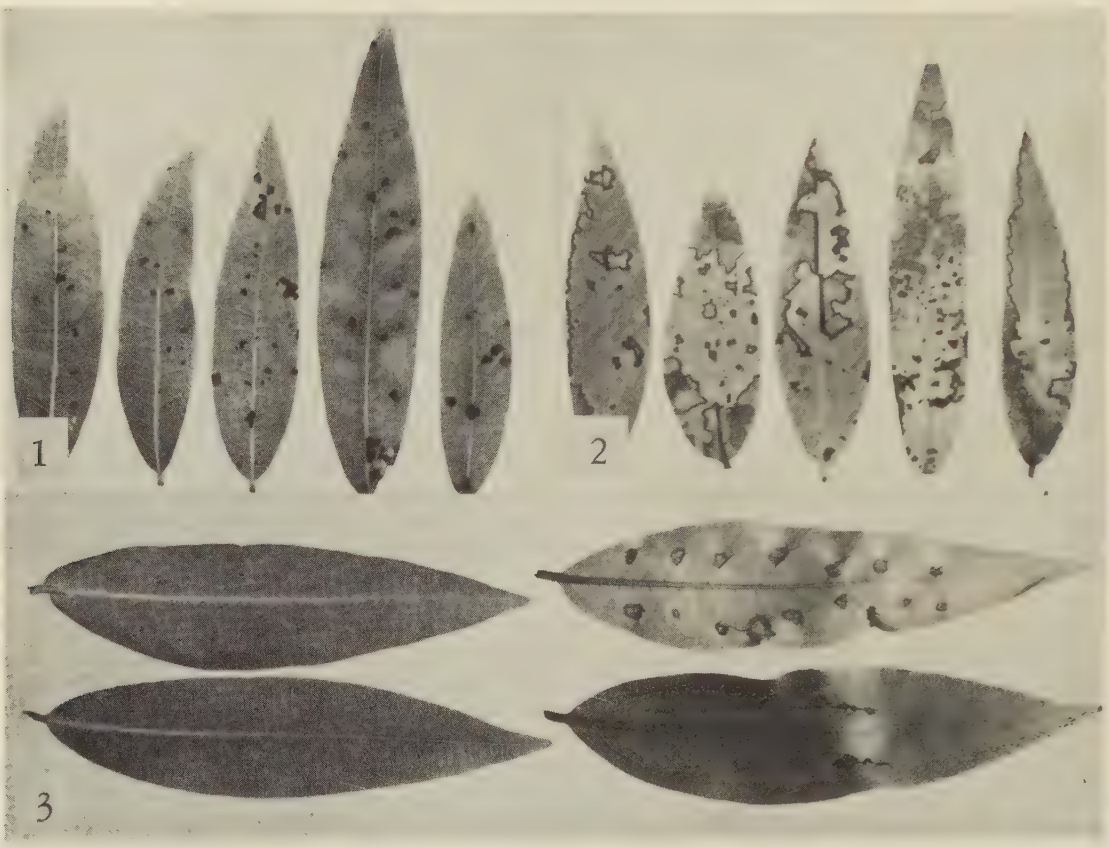

FIGURE 1. Leaves of California-laurel showing angular, black lesions associated with Pseudomonas lauracearum.

FIGURE 2. Irregular brown, necrotic spots associated with Colletotrichum gloeosporioides and Kabatiella phoradendri f. umbellulariae.

FIGURE 3. Wounded laurel leaves inoculated with Colletotrichum gloeosporioides. The leaves on the right are young, current season's leaves showing lesions caused by C. gloeosporioides. The leaves on the left are year-old leaves showing no lesions.

INOCULATIONS

The pathogenicity of P. lauracearum and K. phoradendri f. umbellulariae has been established by Harvey (1, 2). The only known report of C. gloeosporioides on U. californica is that of Hennings in 1898 (3). He found the fungus fruiting on blighted shoots of laurel growing in a greenhouse in Germany and described it as a new species, Fusarium allescherianum. This name has since been reduced to synonymy with C. gloeosporioides (4). Henning's original description was not accompanied by experimental evidence of pathogenicity. Therefore, inoculations were made with potted laurel seedlings to test pathogenicity. The seedlings were sprayed with a conidial suspension prepared from single-spore cultures grown on PDA. The seedlings were then placed in a mist chamber for 1 week and transferred to greenhouse benches. Young leaves that were not fully expanded were blackened and killed within 2 weeks. Young leaves that were fully expanded and year-old leaves were unaffected. Control seedlings sprayed with sterile water remained healthy. The fungus was readily re-isolated from diseased leaves.

Further tests were made to determine whether the fungus could invade older leaves through wounds. A second series of seedlings was inoculated as above, except that some of the leaves on each seedling were punctured in several places with a sterilized needle. Lesions developed around punctures on young, fully-expanded leaves but not on year-old leaves (Fig. 3). No lesions developed on large, unwounded leaves, but again the young leaves that were not fully ex-

panded were blackened and killed. Control seedlings remained healthy. These observations suggest that C. gloeosporioides was able to penetrate small, immature leaves directly, but that as the leaves approached maturity they became resistant to direct penetration. With maturity the leaves apparently became resistant to infection by either direct penetration or by wound invasion.

OBSERVATIONS ON EPIDEMIOLOGY

Blighting of laurel leaves was first called to the attention of the authors in March 1958. At that time the damage was extensive, indicating that the bulk of the infections had occurred during the winter. In order to record the seasonal development of the disease on the 1958 foliage, new shoots on 60 branches were tagged in the fall of 1958. On 45 shoots, the leaves were healthy. On the remaining 15 shoots, one or more leaves showed bacterial lesions. These shoots were examined periodically through July 1959. Only six leaves from the 45 healthy shoots developed bacterial lesions, despite the abundance of older, infected leaves surrounding these shoots. No increase in the number or size of lesions was found on the 15 shoots with infected leaves. Subsequent examination of these tagged shoots and of surrounding trees in April 1960 showed that no new infections had developed during the winter of 1959-60. Trees that were entirely brown in April 1958 had recovered by April 1960. The blighted leaves had been shed and bacterial lesions were found on a few leaves only after intensive searching. No evidence of fungus infection could be found. These observations, coupled with the absence of past records of severe leaf blighting, indicate that such extensive damage to laurels is rare. A search of weather records was made to determine the conditions associated with the sudden, epidemic infection.

Weather records (from Climatological Data, California) for two stations, Muir Woods and Berkeley, were examined. The average precipitation during the months of October through April is 35.73 inches at Muir Woods and 23.68 inches at Berkeley. Obvious deviations from these averages occurred during the winters of 1955-56 and 1957-58. The Muir Woods station recorded 55.07 inches and 51.72 inches respectively, and the Berkeley station recorded 30.02 inches and 35.52 inches respectively during these two winters. These readings are among the highest ever recorded at these stations. The occurrence of two such winters of extreme precipitation within a 3-year period suggested an explanation for the epidemic.

DISCUSSION

The pathogens involved in the described leaf-blight complex occur at very low, endemic levels. Since no extensive damage was reported following the exceptionally wet winter of 1955-56, it appears likely that one such winter is insufficient to induce damaging infection. A marked rise in the level of inoculum undoubtedly occurred, however. Since California-laurel holds its leaves for several years, a high level of inoculum was likely present when the second winter of heavy rainfall occurred in 1957-58, with the result that epidemic leaf-blighting occurred.

The association of the epidemic with the occurrence of two winters of unusually heavy rainfall suggests that similarly severe leaf blighting might be expected when two such winters occur within a 3-year period. The recovery of affected trees indicates that no permanent damage from such an epidemic is likely.

Literature Cited

1. HARVEY, J. M. 1951. An anthracnose disease of Umbellularia californica. Madrono 11: 162-171.
2. HARVEY, J. M. 1952. Bacterial leaf spot of Umbellularia californica. Madrono 11: 195-198.
3. HENNINGS, P. 1898. Die in den Gewachshausen des Berliner botanischen Gartens beobachteten Pilze. Verh. bot. Ver. Prov. Brandenburg 40: 109-177.
4. VON ARX, J. A. 1957. Die Arten der Gattung Colletotrichum Cda. Phytopath. Z. 29: 413-468.

DEPARTMENT OF PLANT PATHOLOGY, UNIVERSITY OF CALIFORNIA,
BERKELEY AND DIVISION OF FOREST DISEASE RESEARCH,
PACIFIC SOUTHWEST FOREST AND RANGE EXPERIMENT STATION,
UNITED STATES DEPARTMENT OF AGRICULTURE, BERKELEY

CONTROL OF STRIPE SMUT ON POA PRATENSIS WITH NABAM

R. J. Lukens and E. M. Stoddard

Stripe smut, caused by Ustilago striiformis (West.) Niessl, can be controlled on Kentucky bluegrass (Poa pratensis) by soil drenches of disodium ethylene bisdithiocarbamate (nabam). On separate plots a 1:400 dilution of a 22% nabam formulation and a 1:2000 dilution of oxyquinoline sulfate were applied to the soil at 1 pint per square foot. Some plots were treated only in October 1959, some only in May 1960, and some were treated on both dates. Three weeks after the spring application, incidence of stripe smut disease was recorded by counting the number of 4-inch squares having disease in 4 x 6-foot plots (Table 1). Oxyquinoline sulfate had little influence on stripe smut, but a drench of nabam reduced the incidence of disease. Fall application of nabam was as effective as spring application.

Table 1. Incidence of stripe smut on Kentucky bluegrass on plots treated with drenches of nabam and oxyquinoline sulfate.

Treatment	Time of application	Number of 4-inch squares having disease in 4 x 6-foot plots
Control		24
Nabam	October and May	5
	October	0
	May	4
Oxyquinoline sulfate	October and May	21
	October	18
	May	16

At another site, Kentucky bluegrass growing in shade and Merion bluegrass growing in open sunlight were uniformly severely infected with Ustilago striiformis. Part of each turf was drenched with a solution of nabam (1:600 dilution of 22% nabam, 1 pint per square foot). In 2 weeks, infected plants in treated areas had a high percentage of healthy growing tips, while infected plants in untreated areas had smutted growing tips. These data and observations suggest nabam as a possible control for stripe smut on Poa pratensis.

DEPARTMENT OF PLANT PATHOLOGY AND BOTANY, THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION, NEW HAVEN

CACAO CANKER IN GUATEMALA INCITED BY CERATOCYSTIS FIMBRIATA

Eugenio Schieber and Oscar Nery Sosa

Branch and stem canker of cacao (Theobroma cacao) incited by Ceratocystis fimbriata Ell. & Halst. (Ceratostomella fimbriata) is reported for the first time in Guatemala. The disease is also known as "mal de machete" in certain countries in South America. First reported from Ecuador in 1918, the disease was later reported from Venezuela, Columbia, Trinidad, Mexico and Costa Rica.

The disease was found affecting Guatemalan clone selections in an experimental plantation located in the Depto. of Retalhuleu in the southern coast of Guatemala.

The affected young trees showed typical symptoms, starting with wilting and yellowing of the leaves. Later the upper leaves turn brown and remain on the branches when dead. Dark brown cankers are visible on branches and stems. After the bark is removed a typical brown to reddish color of the affected wood is observed. Young trees are killed when trunk cankers completely girdle them.

Isolation of the fungus is complicated by a secondary fungus (Diplodia theobromae) sometimes present in the cankers. No insect relation to disease development was found as reported from Columbia.

The pathogen is disseminated through use of the "machete," the common knife used for weeding and pruning. Care in weeding, together with other cultural practices, will help to control this disease. The cacao program in Guatemala is considering the selection of resistant clones for control.

INSTITUTO AGROPECUARIO NACIONAL, GUATEMALA, GUATEMALA C. A.

NEW PINE HOSTS FOR SOUTHERN FUSIFORM RUST

F. F. Jewell[1]

Examination of exotic pines in the arboretum of the Southern Institute of Forest Genetics has revealed additional hosts of the southern fusiform rust, Cronartium fusiforme Hedgc. & Hunt[2].

The trees are 2 to 6 years of age and have been subjected to natural rust infection only. Swollen branch or stem areas considered macroscopically characteristic of C. fusiforme infection were hand-sectioned, stained[3], and examined microscopically for the typical hyphae and haustoria of this fungus.

C. fusiforme galls were confirmed on the following previously unlisted pine hosts: Pinus nigra Arnold, P. cooperi var. ornelasi Martinez, P. pseudostrobus Lindl., and P. torreyana Parry[4]. Caribbean pine (correctly known as P. caribaea Morelet) and south Florida slash pine (P. elliottii var. densa Little & Dorman) were also confirmed as hosts of this rust; previous reports on P. caribaea[2] probably referred to United States slash pine, now correctly known as P. elliottii Engelm. var. elliottii.

SOUTHERN FOREST EXPERIMENT STATION, FOREST SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE

[1]Southern Institute of Forest Genetics, Southern Forest Experiment Station, Forest Service, United States Department of Agriculture. Gulfport, Mississippi.

[2]Previously known hosts were listed by: Hedgcock, G. G., and P. V. Siggers. 1949. A comparison of the pine-oak rusts. U. S. Dept. Agr. Tech. Bull. 978, 30 pp.

[3]Jewell, F. F. 1958. Stain technique for rapid diagnosis of rust in southern pines. Forest Sci. 4: 42-44.

[4]The P. torreyana specimen was supplied by Dr. Charles Hodges, Southeastern Forest Experiment Station.

BOOK REVIEW

"VEGETABLE DISEASES AND THEIR CONTROL," by Charles Chupp and Arden F. Sherf. The Ronald Press Company, New York. v + 693 pages. 1960. Price $12.

Here is a book for commercial vegetable growers, and for backyard gardeners who really care. Teachers and advanced students of plant pathology also will find it useful.

Authors Chupp and Sherf, both Cornell University plant pathologists, have not only drawn on their own knowledge and experience but have painstakingly searched the literature on vegetable diseases to make this book complete, accurate, and practical.

Commercial vegetable farmers will like the way the book is organized. General problems --- seed rot, mineral deficiency diseases, and nematodes --- are given separate chapters. More specific diseases are discussed in chapters related to the crop they attack; sweet corn and cucurbit diseases are examples. In all, 16 chapters are devoted to the diseases of about 40 major vegetables; another chapter takes up the diseases of tropical and little-grown vegetables such as yam, cassava, and chayote.

The authors follow a pattern in reporting on each vegetable disease, first discussing the distribution and occurrence of the disease, then disease symptoms in their various stages, and finally control recommendations. A list of references following each disease discussion is a feature that should be particularly appealing to teachers and students of vegetable diseases.

Some 170 photographs and drawings are used to illustrate this nearly 700-page book. One can believe the authors made as intensive a search for pictures as they did of the scientific literature. Nevertheless, if there is a single weakness, it is the lack of symptom illustrations.

The stature of the authors recommends the book. Charles Chupp, professor emeritus at Cornell, has specialized in vegetable diseases for more than 40 years and is recognized as a world authority on the subject. Before coming to Cornell as Dr. Chupp's successor in 1954, Arden F. Sherf had taught plant pathology and served as extension plant pathologist at the Universities of Nebraska and Iowa State. Vegetable diseases constitute his specialty. --
PAUL R. MILLER